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# On the Internal Skeleton and Musculature of the Crab *Neptunus Sanguinolentus* (Herbst)

BY

M. J. GEORGE *

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ABSTRACT

The endophragmal skeletal system and musculature of the crab *Neptunus sanguinolentus* Herbst are described in comparison with that of the European form *Cancer pagurus,* giving stress to the features which differ. In the endophragmal skeletal system of the species the endosternites of the two sides do not touch the median plate. In the fifth thoracic arthrophragm there is no additional cavity other than the apodemal foramen. In the eighth thoracic arthrophragm there is no division into endopleurite and endosternite, the two halves being separated by the posterior end of the median plate. The skeletal plates of the arthrophragms of the post-oral cephalothoracic region are much reduced to rod like structures of various breadth.

In the muscular system of the crab the occurrence of the muscle connecting the thorax and the abdomen on the dorsal side, "musculus dorsalis superficialis thoraco-abdominalis", which is absent in the blue crab *Callinectes sapidus* is a noteworthy feature which may be of phylogenetic significance.

## *Introduction*

The anatomy of the principal type of European edible crab, *Cancer pagurus* has been studied as early as 1908 by Pearson. The biology, anatomy and affinities of *Corystes cassivelaunus* was the subject of a study by Bouvier (1942). Considerable work has been done on the biology and fisheries of crabs like *Cancer magister* and *Callinectes sapidus.* Among older Indian authors who have worked on different aspects of the biology of the crabs may be mentioned Bhattacharya (1931), Nath (1932), Muthuswamy Iyer (1933), Patwardhan (1934, 1935 and 1937), Reddy (1935, 1937 and 1938) Me-

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non (1937) Chopra (1939) and Nancy Samuel (1945). Recently Menon (1952) studied the bionomics and fishery of *Neptunus sanguinolentus* on the Malabar coast. Prasad and Thampi (1952, 1953, 1954 and 1957) published a series of papers on some aspects of the biology of *Neptunus pelagicus* and some other crabs. The early development of some crabs including *Neptunus sanguinolentus* was studied by Raja Bai Naidu (1951 and 1955). Chhapgar (1957) studied systematics of Bombay crabs. The complete anatomy of no Indian crab has been worked out so far. The present study is intended to give a comprehensive account of the complete anatomy of the common edible crab of the Madras coast, *Neptunus sanguinolentus,* the generic name now revised to *Portunus* (Stephenson and Campbell, 1959), laying particular stress on the points which differ from the European form of *Cancer pagurus* and the following account consists of a part of it dealing with the internal skeleton and musculature of the crab. The account of the other systems will be published in due course.

This work was carried out in 1947-49 at the Madras University Zoology Research Laboratory during the tenure of a research scholarship. It formed part of the thesis approved for the M.Sc. degree of the University, but has since been brought up-to-date. The author wishes to take this opportunity to thank Dr. C. P. Gnanamuthu for his invaluable guidance and criticisms throughout the course of the work. The author is also grateful to the Syndicate of the University for awarding him a studentship during the period of the work.

## *Material and Methods.*

Specimens of the crab from the Madras coast formed the material for the study. The animals for dissection purposes were brought to the laboratory from the beach regularly. For studying the endophragmal skeletal system, the animals were killed and after removal of the appendages put in a dilute solution of caustic potash by which process the soft parts were removed. For the study of the different muscles, the abdomen were dissected from both dorsal and ventral sides.

## *Endophragmal Skeleton* (*Text Figs.* 1-10)

Among the authors who have described the inner skeleton and musculature of Decapoda may be mentioned Schmidt (1915) Bourne (1922), Berkeley (1928), Daniel (1928-32) Cochran (1935)

and Abrahamczik (1942). Bourne has given a description of the inner skeleton of the Rananidae with a discussion on its evolution. Daniel published several papers on the abdominal musculature of several Malacostraca including *Homarus vulgaris, Palinurus vulgaris* and the shore crab *Carcinus maenas.* Cochran gave a complete description of the musculature of all the appendages, abdomen, stomach, and the gastric armature of the blue crab *Callinectes sapidus.* He also gave a short account of the endophragmal skeletal system.

The post-oral cephalothoracic and posterior thoracic somites of the cephalothorax are separated internally by infoldings of the sternal and epimeral skeleton between the somites. The partitions or *arthrophragms,* which are double in formation, form an extremely complicated system of internal skeleton, dividing the body into a series of irregular compartments, affording attachment to the muscles of the somites such as those of the proximal region of the stomach and the other parts of the viscera, and supporting the differrent visceral organs. The dimension and disposition of the various parts of the endophragmal skeleton are correlated with the muscles which have their origin on these.

In most of the Natantia and Reptantia, a sternal canal is formed by the fusion of the sternal plates of the opposite sides in the middle region. But in *Neptunus sanguinolentus* as in almost all other Brachyura, this is absent. The rudiments of the sternal canal are represented by the knobs seen on some of the thoracic endosternites.

The arthrophragms of the post oral cephalothoracic somites and those of the posterior thoracic region, differ much, though built on the same fundamental plan. The fourth thoracic arthrophragm situated in between the fourth and fifth thoracic somites may be described as a type. It is a vertical partition extending from the line of junction between the fourth and fifth thoracic epimera to the line of junction between the fourth and fifth thoracic sterna. Thus it consists of two kinds of plates the *endopleurite* (Text Fig. 1 epl.), growing from the epimera situated on the dorsal and outer side, and the *endosternite* (Text Fig. 1 est.) growing from between the sterna and situated on the ventral and inner side, on each side of the middle line. In the middle region of the posterior thoracic region of the cephalothorax, there is an ingrowth, from the middle suture present on the sterna of that region, which is known as the *median plate* (Text Fig. 1 m.p.). This median plate

extends upto the junction between the fourth and fifth thoracic somites. The endosternites of the two sides of that region do not touch the median plate as described in *Cancer* by Pearson. The endosternite of the fourth arthrophragm stops at about 1 mm. on both sides of the median plate. The other endosternites do not even come so far.

The median border of the endosternite (Text Fig. 2 m.b.s.) stops at about 1 mm. from the median plate. It is vertical and short, about 2 or 2·5 m.m. in height. The sternal border (sb.s) which is in contact with the sternum is slightly convex due to the shape of the sternum and forms the ventral boundary of the endosternite. The articular border (ab.s) starts from the outer end of the sternal border and passes outwards and upwards. It is three fourth the length of the sternal border and is in contact with the arthrodial membrane of the fourth and fifth thoracic appendages. The outer border passes downwards and inwards and fuses with the endopleurite at the two sides, leaving in the middle region, the large apodemal foramen (a.f.) The dorsal border (db.s.) passes upwards and outwards from the median border, forming a curve, with a slight elevation in the middle of the curve (kn) representing the rudiment of the sternal canal as mentioned above.

The dorasal anterior end of the endopleurite is prolonged along the sides of the apodemal foramen to fuse with the end of the outer border of the endopleurite. Four borders can be distinguished in the endopleurite. The inner border is concave and forms the dorsal margin of the apodemal foramen. The two ends fuse with the outer border of the endosternite. The articular border (ab. p) is very much reduced because of the situation of the apodemal foramen towards the outer side and it is only 2 or 3 mm. broad. The epimeral border (eb. p) is very long, being the largest, and is in contact with the fourth and fifth thoracic epimera. The dorsal border (db.p.) is in the form of a right angle, bounding the dorsal free end of the endopleurite.

The endosternite of this arthrophragm is fused on its anterior face with the posterior face of the preceding endopleurite on the inner and ventral side of the apodemal foramen (Text Fig. 2 pp.). Similarly this arthrophragm is fused on its posterior face with the anterior face of the endosternite of the following arthrophragm slightly dorsally to the fusion with the preceding endopleurite (Text Fig. 2 fs.).

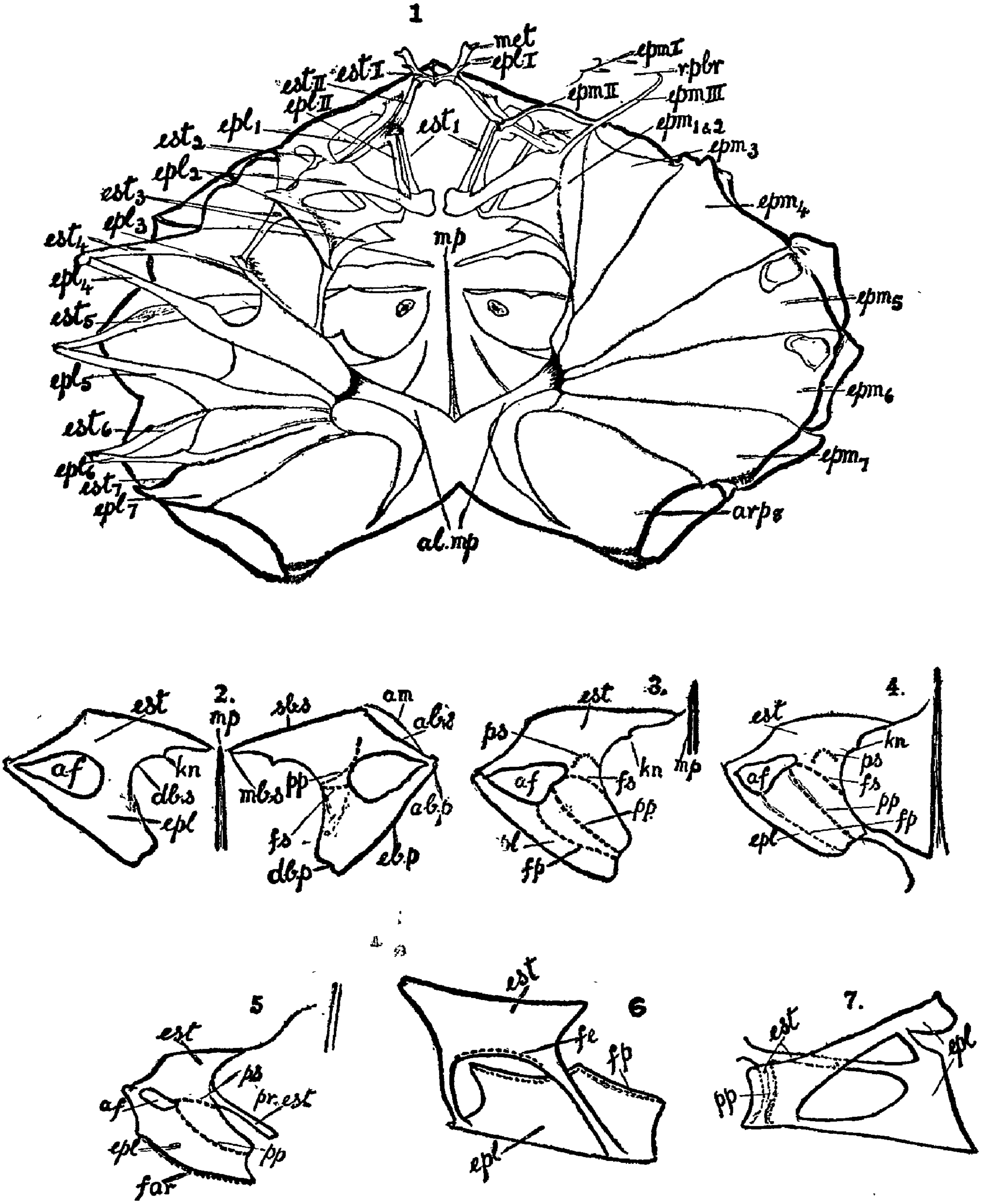
The last five thoracic arthrophragms show a very similar arrangement to the one described above. Although the arthrophragms of the post-oral cephalothoracic region are built up on the same plan some of them are extremely reduced. So the whole of the endophragmal skeleton may be described in two parts, as done by Pearson.

*Posterior thoracic endophragmal system* (Text Figs. 2-5): The arthrophragms of the last five thoracic somites constitute the posterior thoracic arthrophragmal system.

In the region of these somites there is the median plate on the ventral side, as mentioned above. It commences from the fifth thoracic sternum and projects vertically upwards in the middle of the body cavity. Though it starts from the anterior edge of the fifth thoracic sternum its dorsal anterior edge projects forwards and comes slightly in front of the endosternites of the fourth thoracic arthrophragm, so that the anterior vertical edge of the median plate is in a slanting position. There is only a slight difference in height between the posterior and the anterior regions of the median plate, the anterior region being slightly shorter than the posterior region, so that the dorsal edge of the plate is slightly slanting towards the anterior region. The median plate also is made up of two closely applied portions of the exoskeleton, as in other parts of the endophragmal skeleton. The two parts of the median plate are very closely attached and they remain slightly separate ventrally at the suture.

The endosternites of the fourth and fifth arthrophragms are at right angles to the sternum ventrally and dorsally slanting backwards. The posterior arthrophragms are slightly slanting towards the anterior ventrally and slanting backwards dorsally. The endopleurites of these arthrophragms are more or less at right angles to the epimera.

By the fusion of successive endopleurites and endosternites, pleural and sternal muscle chambers are formed. But the sternal muscle chambers are not complete because the endosternites do not extend up to the median plate. Because of the absence of a separate endopleurite and endosternite for the eighth thoracic arthrophragms, only a single chamber, pleurosternal muscle chamber is formed on both sides in that somite and this chamber sends forward an anterior prolongation (Text-Fig. 1 al. mp.) which extends up to the posterior face of the fourth thoracic arthrophragm.




TEXT-FIGS. 1-7.

1. Dorsal view of the endophragmal system. The epimera of the left side have been removed in order to display the endopleurites. 2. Fourth thoracic arthrophragm. 3. Fifth thoracic arthrophragm left side. 4. Sixth thoracic arthrophragm left side. 5. Seventh thoracic arthrophragm left side. 6 Third thoracic arthrophragm left side. 7. Second thoracic arthrophragm left side.

*The fourth thoracic arthrophragm* (Text-Fig. 2) has already been described.

*The fifth thoracic arthrophragm* (Text-Fig. 3): This is situated between the fifth and sixth thoracic somites. The sternal border of the endosternite starts at about 2 mm. on both sides of the median plate. The apodemal foramen is of the same size as in the previous arthrophragm. There is no additional cavity other than the apodemal foramen unlike the description of Pearson (1908).

The endosternite is vertical with the dorsal margin slightly tilted backwards. The ventral region of the endopleurite in connection with the endosternite is more or less horizontal and the dorsal region in connection with the epimera is vertical. A slight elevation on the inner dorsal border of the endosternite (kn) as in that of the previous one may be taken to represent the rudiment of the sternal canal of the Macrura as mentioned above.

In this arthrophragm the fusion of the endosternite and the endopleurite, with the preceding and following endosternite and endopleurite takes place separately. The anterior face of the endosternite fuses with the posterior face of the preceding one at the portion inside the inner and anterior corner of the apodemal foramen ' (ps.) The posterior face of the endosternite fuses with the anterior face of the following one slightly above the place of fusion with preceding endosternite (fs.). Similarly the endopleurite of this arthrophragm fuses anteriorly and posteriorly with the preceding and following endopleurites respectively at the region of the endopleurite on the inner side of the posterior dorsal corner of the apodemal foramen (pp & fp.)

*The sixth thoracic arthrophragm* (Text-Fig 4): This is situated in between the sixth and seventh thoracic somites. The endosternites of this arthrophragm starts far apart from the median plate about 5 or 6 mm. towards the sides. So the sternal border is shorter than those of the previous arthrophragms. The middle portion of the endosternite is slanting forwards and the dorsal portion backwards, so that it is convex. The arrangement of the endopleurite and the attachment of the preceding and following endopleurites and endosternites (pp, fp, ps & fs.) are similar to those of the fifth thoracic arthrophragm. The knob representing the sternal canal is very much reduced leaving only a very small elevation. No other foramen except the apodemal foramen is pre-

sent and the dorsal border is not fused with the anterior edge of the last arthrophragm unlike Pearson's description.

*The seventh thoracic arthrophragm* (Text-Fig. 5) arises between the seventh and eighth thoracic somites. The whole arthrophragm is convex. The endosternite is in a slanting position and starts at about the same distance from the median plate as the previous one. But, for half the length of the sternal border, it is represented only by a thick line and then only the vertical portion begins. So the endosternite is much narrower than the previous ones. The dorsal border of the endosternite is far apart from the median plate than those of the previous ones. Even the small elevation found on the dorsal border of the previous endosternite, representing the sternal canal is absent here. But at the place of that, there starts a narrow process (pr. est.) which leads forwards and upwards and joins the dorsal border of the sixth endosternite. The apodemal foramen is much smaller.

The endopleurite is more horizontal and the whole arthrophragm is thus convex surrounding the pleurosternal muscle chamber.

In this arthrophragm the fusion with the following arthrophragm takes place only at one place, there being no separate endosternite and endopleurite for the eighth thoracic arthrophragm. The fusion with the eighth arthrophrgam takes place at the epimeral border of the endopleurite of this arthrophragm (f. ar.). The anterior face of the endopleurite of this arthrophragm fuses with the posterior face of the previous endopleurite at a line on the inside of the inner corner of the apodemal foramen (pp). It also fuses with the previous endosternite slightly ventral to the fusion with the endopleurite. (p.s.)

*The eighth thoracic arthrophragm* (Tex-Fig. 1. ar. p.8) : In this arthrophragm there is no division into endopleurite and endosternite as already mentioned. The two halves are separated by the posterior dorsal end of the median plate. The dorsal margin of the median plate at the region of the pleurosternal chamber is 4 or 5 mm. broad and is concave. The broad portion ends with the anterolateral prolongation which fuses with the posterior face of the fourth thoracic arthrophragm. Beyond the starting place of these processes the dorsal border of the median plate is narrow, being of the same thickness as the plate itself.

The eighth arthrophragm is more or less horizontal with the posterior end slightly downwards. This is the 'sella turcica' of Milne Edwards. In between the broad posterior region and the anterolateral prolongation of the median plate, and the inner ends of the eighth thoracic arthrophragms and the fifth, sixth and seventh endopleurites, there is a foramen on each side.

(2) *Anterior post-oral endophragmal system*: This system consists of the first three thoracic arthrophragms and the two post-oral cephalic arthrophragms.

There is great difference between the arthrophragms of this system and those of the posterior thoracic endophragmal system. The shape and size of these arthrophragms depend upon the nature of the epimera and the sterna of the respective somites. The epimera of the anterior thoracic somites are fused together so that the endopleurites arising from these are naturally much reduced. Similarly the anterior thoracic sterna are also fused together resulting in the extreme reduction of the endosternites. Another reason for the difference of this system from the posterior thoracic endophragmal system is the narrowness of the region and the absence of the median plate. Thus due to all these reasons the skeletal plates of this system are much reduced to rod-like structures of various breadth. The arrangement of these rod-like structures and plates due to the fusion of the sterna and epimera is such that in some cases their homology is much disguised. In most cases the fusion between the preceding and following arthrophragms, as in the case of the posterior thoracic endophragmal system, is carried out by means of thin rods.

***The third thoracic arthrophragm*** (Text-Fig. 6): This lies between the third and fourth thoracic somites. The endosternite is much reduced and thus different from the typical one described above. It commences merely from the outer edge of the sternum in between the third and fourth thoracic somites. Only the outer edges of the endosternites of the two sides touch the sternum so that the two endosternites are separated from each other by the entire width of the sternum. The inner portion of the endosternite projects into the cavity of the thorax. It is broad, more or less, rectangular plate, placed in a backwardly slanting position. The inner corners do not come near the middle region of the body. The outer border of the endosternite fuses with the epimeron (f.e.) and from the two corners arise two rod-shaped structures which

pass backwards and join the endopleurite near the epimeral border at the two sides.

The endopleurite is also rectangular and arises between the third and fourth epimera. It is placed more or less vertical, the ventral border slightly slanting backwards and there it fuses with the following endopleurite (f.p.).

The endopleurites and endosternites of this arthrophragm are not situated in the same line and their connections between each other are only the two rod-like structures.

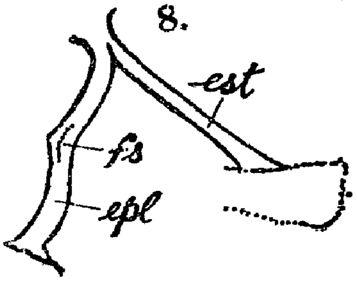
*The second thoracic arthrophragm* (Text-Fig. 7): The endosternite of this arthrophragm is reduced to two rod-shaped structures arising from the upturned edge of the sternum. One of the rods passes inwards and backwards and fuses with the endopleurite at the anterior region of the inner border of the foramen in the same. The other rod representing the articular border passes outwards and backwards and slightly upwards to fuse with the endopleurite at the outer corner near its epimeral border. At about one third of the length of the outer rod of the endosternite from the starting place at the sternum, it fuses with the rod-like endopleurite (pp.) of the first thoracic arthrophragm for a length of about 1 mm. and then separates.

The endopleurite consists of two parts, an inner posterior triangular plate and an anterior spade-shaped structure. At its inner corner inner to the portions where the inner rod of the endosternite fuses with it, the triangular region is joined by a short process with the posterior inner margin of the spade-shaped structure, which is situated transversely in the body, so that the wider inner ends of this part of the two sides come together in the centre. At the outer ventral corner also these two regions join together, forming a foramen in the middle.

*The first thoracic arthrophragm* (Text-Fig. 8) : The endosternite of this is rod-like and arises from the upturned border of the sternum. It passes backwards, inwards and slightly upwards and fuses with the inner anterior margin of the spade-shaped part of the endopleurite of the following arthrophragm.

The endopleurite is also rod-shaped and starts from the outer anterior corner of the fused first and second thoracic epimeral plate. It passes inwards, forwards and slightly downwards, parallel to the outer rod of the second thoracic endosternite and fuses

8.

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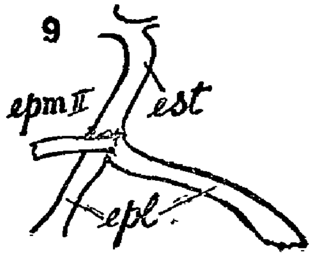


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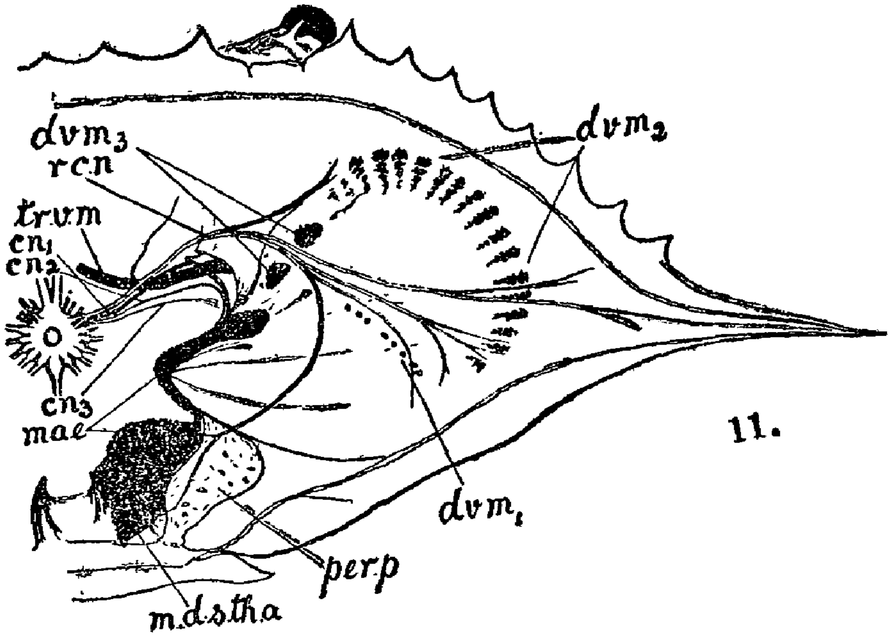


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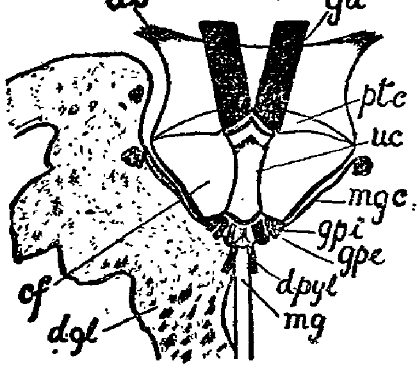


TEXT-FIGS. 8-12.

8. First thoracic arthrophragm left side. 9. Last cephalic arthrophragm left side. 10. First cephalic arthrophragm. 11. Dissection of the right side from the dorsal side showing the dorso-ventral muscles and the muscular attractor epimerales. 12. Dorsal view of the fore-gut showing the muscles.

with the endosternite very near its starting point. In the middle portion it fuses with the outer rod of the second thoracic endosternite (f.s.).

*The last cephalic arthrophragm* (Text-Fig. 9). The endosternites of the two post-oral cephalic arthrophragms are fused together. Even then they can be distinguished by a suture in between them. The fused endosternities are situated in the anterior-most region of the sternum. They pass outwards and backwards parallel to the first thoracic endopleurite for a distance of about 2 mm. Then the two separate and the endosternite of the last cephalic arthrophragm passes backwards in the same direction and joins the two rods of the endopleurite of the same.

The endopleurite is represented by two rod-like structures. One rod is in continuation with the endosternite and parallel to the endopleurite of the following arthrophragm, and joins the outer anterior corner of the fused first and second thoracic epimera. The other is a thin rod-like plate passing backwards and upwards from the place of fusion of the endosternite and the other rod of the endopleurite, and joining the inner and dorsal side of the spade-shaped part of the endopleurite of the second thoracic arthrophragm. The portion which is fused is slightly broader.

*The first post-oral cephalic arthrophragm* (Text-Fig. 10): From the portion where the endosternite of this arthrophragm separates from that of the following, it has a process directed upwards at each side (met.). These two processes form the skeleton of the metastoma or posterior lip of the mouth. The dorsal posterior articulation of the mandible is articulated at the tip of this process.

The endopleurite is a rudimentary, thin and spine-like process from the anterior surface of the endosternite, directed anteriorly above .the endosternite of the following arthrophragm. Dorsal to this process, passes backwards the long tendon of the internal adductor muscle of the mandible, so that it acts as a hinge.

## *Muscular System* (Text-Figs. 11-17)

Pearson does not describe the trunk muscles of *Cancer* However, the complete fusion of the segments of the head and body and the formation of the cephalo-thorax, encased in a hard shield, the carapace, resulting in the absence of the complex intersegmental muscles, which in crustaceans like the shrimp, cray fish etc., give a high degree of flexibility to

the movements of the body, render the study of trunk muscles of special interest. Of all these complicated muscles which are encountered in the cray fish and the shrimp, only one muscle has its counter-part in the crab. This is a pair of *musculus attractor epimeralis* (Text-Fig. 11. m.a.e.) which Cochran (1935) has also described in the blue crab, *Callinectes sapidus.* The short fibres of each muscle run between and unite the inner posterior corner of the fused thoracic epimera with the carapace, between the branchial and cardiac regions.

As mentioned before, continuous with the inner posterior corner of the fused epimera there is a slightly calcareous small triangular plate with its base forwards, placed in a more or less vertical position. The membranous roof of the branchial chambers is continuous with this plate. Anteriorly the muscle has its fibres attached to this triangular plate and it extends posteriorly beyond the corner of the fused epimera, upto the inner region of the eighth thoracic arthropragm, at the region inner to the pericardial pouch. The fibres of the muscle are present on the eighth thoracic arthrophragm on whole of the region inner to the pericardial pouch. Thus the *musculus attractor epimeralis* forms a thick bushy line of short muscular strands extending from the anterior edge of the triangular plate, anterior to the inner corner of the fused thoracic epimera, to the eighth thoracic arthrophragm in a zigzag way. The muscular strands are very short, about 1 or 1·5 mm. in length.

*The dorso-ventral muscles* (Text-Fig. 11). These are muscles extending from the roof of the branchial chamber to the carapace. Some of the muscles are small and others larger. Three sets of muscles can be made out.

The posterior set of muscles (d.v.m.[1]) consists of eight extremely small muscular strands situated in a line in an anteroposterior direction parallel to the antero-lateral border of the carapace. These muscles are very short, the distance between the roof of the branchial chamber and the carapace at this region being very small, the two almost touching each other.

The second and largest set of muscular strands (d.v.m2) is situated along the antero-lateral and lateral borders of the branchial chamber. These muscles are fairly long, the distance between the branchial roof and the carapace at this region being great. In between these two is situated the outward prolongation of the hepatopancreas and the reproductive glands. So the muscular strands

go up from the roof of the branchial chamber, in between the lobules of the hepatopancreas. There are 15 to 20 muscular strips arranged in a row along the outer margin of the branchial roof. Each muscular strip is made of several strands of which the outermost is the biggest and gradually become smaller towards the inside.

The third set (d.v.m. 3) is situated on the inner anterior region of the branchial roof. There are only two muscular strands. These are in continuous line with the second set of muscles, but are bigger in size than the latter. They start from the branchial roof at the region dorsal to the inner ends of the second and third gills and are inserted on the carapace just on the anterior outer side of the region where the internal adductor muscles of the mandible are inserted. These are the biggest of the dorso-ventral muscles in *Neptunus sanguinolentus.*

There is another pair of transparent muscles (tr.v.m.) connected with the branchial roof, but not inserted on the carapace. This muscle is attached on the inner side of the antero-lateral edge of the branchial roof, dorsal to the tip of the second gill. It passes along with the recurrent cutaneous nerve (r.c.n.), slightly upwards, inwards and forwards, and is inserted at the posterior outer end of a small, flat transverse tendon with a foramen in the centre, which is situated dorsal to the inner middle ends of the spade-shaped part of the endopleurite of the second thoracic arthrophragm. This muscle is flat and transparent. The function of this muscle may probably be to draw the anterior region of the roof of the branchial chamber inwards in a lateral direction so that the cavity inside the chamber is increased sideways, by its contraction.

The exact significance of these movements is difficult to understand. The contraction and relaxation of the first three sets of the dorso-ventral muscles and also of the last one will effect a corresponding increase or decrease in the volume of the branchial chamber, the membranous roof being pulled up and down and also sideways.

When a live crab is taken out of water and held outside in the air for sometime, a movement can be noticed beneath the carapace on both sides at the region of the branchiae along with the bubbling noise brought about by the movement of the slight amount of water and air present inside the branchial chamber. This can be noted more clearly if the crab is a soft one. Thus it is clear that a movement of the branchial roof is taking place and this can be

brought about only by the dorso-ventral muscles. Pearson is of opinion that these actions may be of supreme importance in connection with the branchial chamber, either as a current regulator or as an accessory current producing organ.

The muscles which are attached to the carapace produce an impression on it according to the shape and size of the muscular strands.

*Muscles of the appendages*:

A preliminary examination of the masculature of the appendages of *Neptunus sanguinolentus* showed that it is identical with the description given for *Cancer* by pearson (1908) and for *Callinectes sapidus* by Cochran (1935). However, since the last peraeopod is modified into a flat oar-like appendage in adaptation with the mode of life, and shows some difference in the size and number of muscles in *Neptunus* its musculature may be described. *Muscles of the last peraeopod* (Text-Fig. 13) : Both extensor and flexor muscles are present for the meropodite, though according to Pearson only the extensor muscle is present for the meropodites of all the legs.

All the muscles of the coxa and basiischium are situated inside the pleurosternal muscle chamber below the eighth thoracic arthrophragm.

The tendon of the dorsal extensor (d.ext.c.) muscle of the coxopodite is inserted at the middle region of its dorsal side and the ventral extensor (v.ext.c.) is inserted slightly posterior to it. The flexor of the coxopodite is inserted at the middle of the ventral side. The dorsal extensor muscle is the largest of the three.

The movement of the coxa of the chela is in a horizontal direction. In each of the succeeding walking legs, the coxa is more tilted, till in the last walking leg the hinges are anterior and posterior so that the coxa swings in a more or less vertical plane slightly tilted anteriorly. The additional extensor muscle aids in this movement. *Muscles of the foregut*: (Text-Fig. 12)

Both extrinsic and intrinsic muscles are present. *Extrinsic muscles*: The anterior gastric muscles (Text-Fig. 12 ga) are the stoutest, and longest of the muscles of the foregut and are placed in a slightly oblique position, the anterior end slightly towards the outside. Each muscle is more than twice as long as it is broad. The inner posterior muscles (g.p.i.) arise from the two

calcareous rod-like structures, situated at the basal portion of the gastric region of the carapace, as mentioned previously. The muscle is broader near the attachment to pyloric ossicle.

The outer *posterior gastric muscles* (g: p.e.) arise from the under side of the gastric region of the carapace, a little in front and towards the sides of the two rod-like structures from which start the inner posterior gastric muscles and the dorsal pyloric dilator muscles.

*The posterolateral dilator muscles* start from the roof of the pre-branchial chamber. The point of insertion on the foregut is thrice as broad as the origin of the muscle. Each one is 10 mm. long in a crab measuring 8·5 cm. in breadth without including the lateral spines.

Only one pair of ventral pyloric dilator muscles is present. *Intrinsic muscles*: The arrangement of the three pairs of *lateral cardiac muscles* is different from that of *Cancer* because of the position of the pyloric foregut, being at right angles to the cardiac foregut. At one end all the three arise from the edge of the infero-lateral cardiac ossicle. The three muscles here may therefore be distinguished as anterior, middle and posterior lateral cardiac muscles instead of the upper, middle and lower described by Pearson. The anterior muscle extends from the middle portion of the infero-lateral cardiac ossicle to the anterior outer edge of the postero-lateral cardiac plate. The middle one is made up of two strands. Both of these arise from the upper edge of the infero-lateral cardiac ossicle and are inserted on the pectineal and zygocardiac ossicles. The posterior muscles arises below the middle one and is inserted on the posterior portion of the zygocardiac ossicle.

There are several pairs of lateral pyloric muscles. Two pairs are very distinct, extending from the end of the post pectineal ossicle to the base of the dorsal pyloric dilator muscles.

On the sides of the posterior wall of the cardiac foregut, occurs a pair of muscles extending from the base of the inner posterior gastric muscles to the base of the dorsal pyloric dilator muscles. These are absent in *Cancer* as Pearson does not describe them.

*Muscles of the abdomen* (Text-Figs. 14-17):

Compared to the abdomen of the cray fish, lobster, shrimp, etc., which are extremely useful in swimming, the

abdomen of *Neptunus sanguinolentus* and other Brachyura, at least in the male, indicates a condition of partial rigidity, as the third, fourth and fifth segments in that sex are immovably fused although the former segmentation is still partly maintained in its musculature. The female adbomen has six distinct segments with all the muscles developed.

Pearson (1908) in his memoir on *Cancer pagurus* asserts that "on account of the third, fourth and fifth somites being fused together, the abdominal muscles of the male abdomen differ from those of the female." In *Neptunus sanguinolentus* there is no such difference between the male and female abdomens as regards the number of extensor muscles, the only difference being in the size and shape which can be explained to be due to the difference in shape of the male and female abdomens. Daniel (1932) has stated after examination of the abdomen of *Cancer pagurus* that Pearson was partly in error and that his statement that "there are no extensors between the third and fourth and also between the fourth and fifth somites", is not true. According to him three pairs of extensor muscles are present in the fused third, fourth and fifth somites and there is no difference between the male and female in this feature. He has described a similar arrangement in the shore crab *Carcinus maenas*. Cochran (1935) also did not find any difference between the number of dorsal extensor muscles due to the fusion of segments in the male and the female in the blue crab, *Callinectes sapidus*.

Each abdominal segment possesses what are essentially paired dorsal and ventral muscles, although there has been a certain amount of fusion between the members of some of the pairs.

*Musculus ventralis superficialis thoraco-abdominalis* (Text-Fig. 16 & 17 m.v.s.th.a.) ("Flexor" of the first somite of Pearson). This muscle arises from the middle concave region, from where the median plate starts, of the posterior surface of the last thoracic somite and is inserted in front of the line separating the first and second sterna of the abdomen. As in *Callinectes sapidus*, described by Cochran (1935) this is the only trace in the crab of the ventral superficial thoracic muscles, which are very prominent between the highly movable body segments in *Astacus, Pandalus*, etc. Although the muscle is paired, as are all the other abdominal muscles, the strands of the two sides are so close to each other toward the middle line that they appear as one median symmetrical bundle of muscle fibres. The strands towards the middle line are longer than those

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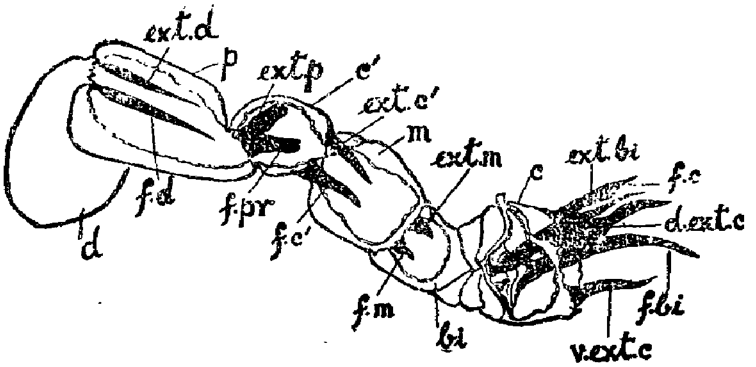


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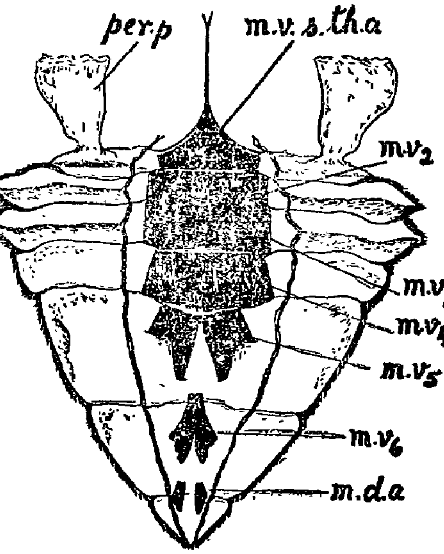


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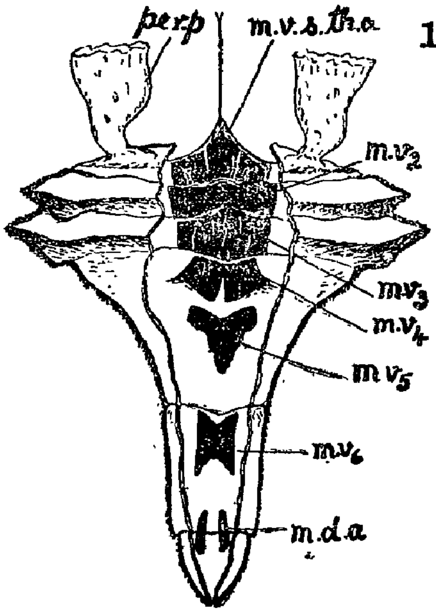


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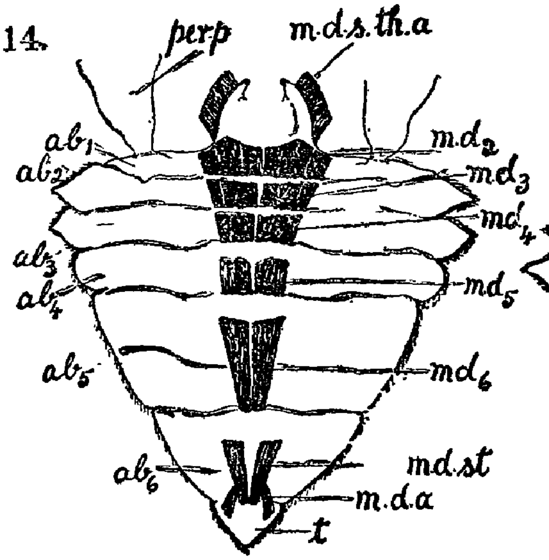


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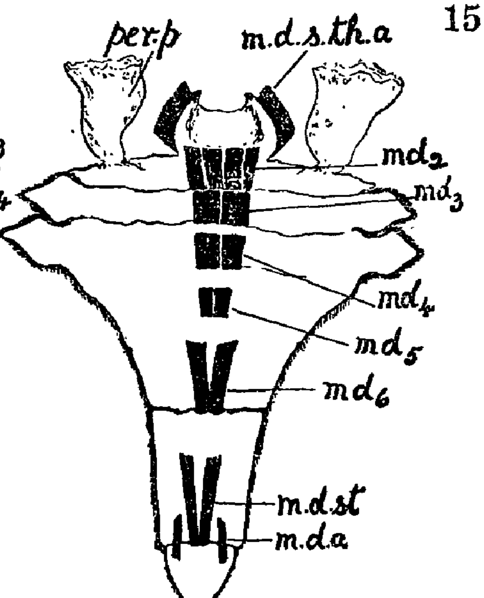


Text-Figs. 13-17.

13. Dorsal view of the last peraeopod (right); the dorsal wall of each segment excepting the dactylopodite has been cut out and soft parts removed

at the sides, because of the triangular shape of the posterior middle region of the last thoracic somite. This muscle helps to pull abdomen towards the thorax.

*Musculi ventrales superficiales abdominis* (Text-Figs. 16 & 17 m.v. 2-6) : These muscles are arranged regularly on the ventral side in accordance with the segmentation of the abdomen. The fusion of the third, fourth and fifth somites in the male has not affected the ventral musculature at all. All these muscles in the female except that of the sixth somite are broader than those of the male. The muscles of the first pair arise from just behind the place where the posterior ends of the musculi ventrales superficiales thoraco-abdominales are inserted, from the line separating the first and second abdominal sterna and are inserted on the sclerotised ridge marking the junction between the second and third segments. The muscles of the two sides are confluent on the middle line. The two succeeding pairs in the female are arranged likewise in the corresponding segments. The muscles of the second pair of the male arise from just behind the line separating the second and third abdominal sterna and are inserted on a line in the middle region only, which is in the same place where the line separating the third and fourth abdominal sterna is seen in the female. The third pair of the male starts from behind that line and is inserted on two small cartilage-like thickenings at an angle of 40° to the horizontal on the two sides. The muscles of the two sides are slightly separated in the posterior region of the middle line, although at the anterior region the two muscles are close together. The muscles of the fourth pair of the female start from the middle of the line separating the fourth and fifth abdominal sterna and are inserted on two cartilaginous projections on the two sides in the middle region of the fifth abdominal segment. So these muscles do not extend the whole length of the segment and the posterior ends of the muscles of the two sides are far apart while the anterior ends are closer together. The muscles of the fourth pair of the male do not arise from the place where the previous pair ends but it arises from a slightly sclerotised concave line in the middle region, a little dis-

in order to show the tendons of the muscles. 14. Dissection of the abdomen of the female from the ventral side, showing the dorsal muscles. 15. Dissection of the abdomen of the male from the ventral side, showing the dorsal muscles. 16. Dissection of the abdomen of the female from the dorsal side, showing the ventral muscles. 17. Dissection of the abdomen of the male from the dorsal side, showing the ventral muscles.

tance behind the place where the previous pair is inserted. These muscles also do not extend up to the line separating the fifth and sixth sterna, but are inserted on two convex invaginated lines making an angle of 50° to the horizontal. The muscles of the two sides are not separated and are completely close together at both ends. In the female the fifth and last pair of muscles is much narrower than the others. The anterior end which is narrow arises from the middle of the line separating the fifth and sixth abdominal sterna. The muscles of the two sides arise close together. They diverge out, become broader and are inserted on lines inclined at 60° to the horizontal. In the male also these muscles arises from the same place, but they do so from a concave line. The muscles of the two sides are undivided, run close together, and are inserted on a convex invagination at about the middle region of the sixth segment. As in *Callinectes sapidus* there is no ventral muscle connecting the sixth segment with the telson in either sex in *Neptunus*.

The muscles connected with the opening and widening of the anus, *Musculus dilator ani* (m.d.a.) are present in the telson. The main pair of muscles connected with this is inserted on the ventral side of the telson. A small pair is inserted at the dorsal wall of the telson also. These two pairs of muscles open the anus by their contraction, while elasticity of the anus opposes them.

*Musculus dorsalis superficialis thoraco-abdominalis* (Text-Figs 14 and 15 m.d.s.th.a.) ("Extensor" of the first somite of Pearson): One pair. Each arises as a broad band of muscle made up of numerous muscular strands from the postero-lateral edge of the horizontal plate of the arthrophragm of the eighth thoracic somite. It passes inwards and backwards for a short distance and is inserted on the side of the anterior rectangular portion of the tergum of the first abdominal somite which projects into the posterior region of the thorax in between the horizontal plates of the arthrophragms of the eighth thoracic somite. Cochran (1935) in his description about the abdominal musculature of the blue crab, *Callinectes sapidus* has stated that this muscle connecting the thorax and abdomen does not occur either in *Pandalus* or in *Callinectes,* while it is present in *Astacus*. This muscle might have an action in the reverse direction as the muscle connecting the thorax and the abdomen on the ventral side. When this contracts the rectangular portion is pulled forwards. Consequently the tip of the abdomen will be pulled outwards, thus widening the distance between the abdomen and the thorax, the portion of the second and third abdo-

minal somites touching the posterior end of the thorax, when in natural condition, acting as a lever arrangement.

*Musculi dorsales superficiales abdominis*: (m.d. 2-6) (Extensors 2-6 of Pearson).

*Female* : The first pair of dorsal superficial muscles arises at the base of the rectangular membrane, mentioned above, and is inserted on a calcareous line in the middle region of the line separating the first and second abdominal terga. Each muscle of this pair is in several parts lying side by side and the muscles of the two sides are distinct. The strands of the muscle are short and broad. The second pair rises from a calcareous line 0.5 mm. behind the line on which the previous muscles are inserted, and are inserted on a sclerotized ridge in the middle of the line separating the second and third terga. The third pair rises 1 mm. behind the posterior end of the previous pair and is inserted in between the third and fourth terga. The fourth pair arises 2 or 2·5 mm. behind the previous pair and is inserted in between the fourth and fifth terga. The fifth and last pair arises from a slightly calcareous ridge about 3 or 4 mm. behind the posterior end of the previous pair. The two muscles start separately on the sides, leaving a gap in between. They converge as they pass posteriorly and are inserted in the middle of the line separating the fifth and sixth terga. All these muscles are broader at the anterior end, and are inserted at both ends on sclerotized ridges.

*Male* : In the male the first pair of muscles are narrow and longer. The second pair of muscles are also narrow and longer than that of the female. Although there is a fusion between the third, fourth and fifth abdominal somites, the number of muscles remain the same as in the female, unlike in *Cancer* and *Callinectes*. All the three pairs of muscles of these somites are present at the respective places as found in the female. However, the muscles are narrower and very transparent.

*Musculus dorsalis superficialis telsonis* (m.d.st.) : This is the last pair of dorsal superficial muscle. The muscles are long and slender with anterior ends slightly broader. The anterior end arises from the anterior half of the sixth tergum on both sides of the middle line. The muscles become slightly narrower as they pass posteriorly and are inserted on the anterior border of the telson, which receives all its power of motion from these muscles, as no flexors exist in either sex for the telson. The muscles of the male are narrower than those of the female.

## EXPLANATION OF TEXT-FIGS.

$ab_{1-6}$—Abdominal somites; ab.p.—Articular border of endopleurite; ab.s—Articular border of endosternite; af—Apodemal foramen; al.mp.—Antero-lateral prolongation of the median plate; am—Arthrodial membrane; arp8—Eighth thoracic arthrophragm; as—Upper anterior dialator muscles; bi—Basi ischium; c—Coxopodite; C′—Carpopodite; cf—Cardiac foregut; $cn_{1-3}$—Cardiac nerves 1-3; d—Dactylopodite; dbp—Dorsal border of endopleurite; dbs—Dorsal border of endosternite; d.ext.c—Tendon of the dorsal extensor muscle of the coxopodite; dgl—Digestive gland; dpyl—Dorsal pyloric muscle; $dvm_{1-8}$—dorsoventral muscles; ebp—Epimeral border of the endopleurite; epl—Endopleurite; $epl_{I}$—Endopleurite of the first cephalic arthrophragm; $epl_{II}$—Endopleurite of the last cephalic arthrophragm; $epl_{1-7}$—Endopleurites of the first to seventh thoracic arthrophragms; $epm_{I-III}$—Epimera of the cephalic somites 1-3; $epm_{1-7}$—Epimera of the thoracic somites 1-7; est—Endosternite; $est_{I}$—Endosternite of the first tharacic arthrophragm; $est_{II}$—Endosternite of the last thoracic arthrophragm; $est_{1-7}$—Endosternites of the thoracic somites 1-7; ext.bi—Tendon of the extensor muscle of the basi ischium; ext.c′—Tendon of the extensor muscle of the carpopodite; ext.d—Tendon of the extensor muscle of the dactylopodite; ext.m—Tendon of the extensor muscle of the meropodite; ext.p—Tendon of the extensor muscle of the propodite; f.ar—Line of fusion with the 8th thoracic arthrophragm; f.bi—Tendon of the flexor muscle of the basi ischium; f.c—Tendon of the flexor muscle of the coxopodite; f.c′—Tendon of the flexor muscle of the carpodite; f.d—Tendon of the flexor muscle of the dactylopodite; f.e—Line of fusion of the endosternite with the epimera; f.m—Tendon of the flexor muscle of the meropodite; f.pr—Tendon of the flexor muscle of the propodite; f.p—Line of fusion with the following endopleurite; f.s—Line of fusion with the following endosternite; g.a—Anterior gastric muscle; g.p.e—External posterior gastric muscle; g.p.i—Internal posterior gastric muscle; kn—Knob representing the sternal canal; m—Meropodite; m.a.e—Musculus attractor epimeralis; m.b.s—Median border of the endosternite; $m.d_{2-6}$—Musculi dorsales superficialis abdominis; mda—Musculus dilator ani; md.st—Musculus dorsales superficialis telsonis; md.sth.a—Musculus dorsales superficialis thoraco abdominalis; met—Metastoma; mg.—Midgut; mgc—Midgut caecum; mp—Median plate; $mv_{2-6}$—Musculi ventrales superficialis abdominis; m.v.s.th.a—Musculus ventrales superficales thoraco abdo-abdominalis; p—Protopodite; per.p—Pericardial pouch; pp—Line of fusion with the preceding endopleurite; pr.est—process joining the 7th and 6th endosternite; ps—Line of fusion with the preceding endosternite; pt.c—Pterocardiac ossicle; r.c.n—Recurrent cutaneous nerve; r.pbr—Roof of the prebranchial chamber; sbs—Sternal border of the endosternite; t—Telson; tr.v.m—Muscle connectd with the branchial roof not connected on the carpace dorsally; uc—Urocardiac ossicle; v.ext.c—Tendon of the ventral extensor muscle of the coxopodite.

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# The Anatomy of the Crab *Neptunus Sanguinolentus* (Herbst)

## *Part* II. *Nervous System and Sense Organs*

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ABSTRACT

The nervous system and sense organs of the crab *Neptunus sanguinolentus* Herbst are described. The presence of three cardiac nerves arising from the ventral nerve mass and the pericardial nerve plexus are noteworthy features in the nervous system of this crab.

The occurrence of both hexagonal and tetragonal facets in the cuticular cornea of the eye is also interesting.

### *Introduction*

The present contribution is a part of the study of the complete anatomy of the common edible crab of the Madras coast, *Neptunus sanguinolentus* (the generic name revised to *Portunus* according to the latest nomenclature), and is in continuation of the account of the internal skeleton and musculature already published. As in the earlier paper, stress is laid on the points which differ from the European form *Cancer pagurus* described by Pearson (1908).

I wish to thank Dr. C. P. Gnanamuthu for his guidance and help and the Syndicate of the Madras University for the award of a studentship during the period of the work.

### *Nervous System*

The description of the nervous system of the shore crab, *Carcinus maenas* was given by Bethe as early as in 1895. Among other authors who have later contributed to the knowledge of the nervous system and sense organs of Decapods may be mentioned, Hensen (1863), Lemoine (1868), Parker (1890 & 1891), Jolyet and Viallanes (1892), Connant and Clarke (1896), Prentiss (1901), Carl-

* Part of a thesis approved for the M.Sc. degree of the Madras University.

son (1905), Police (1908), Hilton (1914), Alexandrowicz (1932) and Hanstrom (1933, 1934). Jolyet and Viallanes (1892) made a study of the innervation of the heart of *Carcinus maenas* and described the pericardial plexus, which was confirmed by Connant and Clarke who worked on the blue crab, *Callinectes sapidus.* Both Carlson and Police confirmed their opinion. Alexandrowicz also studied the innervation of the Crustacean heart muscle and recorded one nerve going to the dorsal surface of the heart from the side. The histology, development and innervation of the compound eyes of lobster and other Crustacea were extensively studied by Parker. Hensen was one of the first to study the auditory organ or otocyst of Decapoda. Prentiss later described the structure, development and function of the otocyst.

The nervous system of *Neptunus sanguinolentus* is fundamentally the same as described by Bethe (1895), Pearson (1908) and others. But one important difference from the description of the nervous system of *Cancer pagurus,* as given by Pearson, is the presence of four additional nerves arising from the ventral nerve mass.

The system consists mainly of two nerve masses, an anterior, dorsal, fused preoral ganglion or brain, which innervates the eyes, the antennules, antennae and the anterior region of the integument below the carapace, and a posterior ventral fused ganglia of the post-oral region innervating the rest of the parts of the body, connected together by a pair of commissures passing around the oesophagus.

*The Brain* : The brain or the cerebral ganglion which is situated dorsal to the middle of the anterior region of the epistoma is roughly rectangular in shape with the lateral sides rounded up. The nerve cells of the anterior region of the brain supply the eyes and the optic penducles, with fibres, and of the posterior half supply the antennules, antennae, the antennal sense organs and the anterior region of the integument. The neuropiles and cells are arranged in the same way as in *Cancer.*

The nerves arising from the brain are a pair of optic, oculomotor, antennulary, tegumentary and antennary nerves and two commissures connecting it with the ventral nerve mass.

Each of the *optic nerves* (Text-Fig. 1 on.) arises from the anterolateral corner of the brain. It runs outwards, forwards and

slightly upwards, so that it is only slightly slanting, about 20° or 25° to the horizontal line, and passes into the optic peduncle. At the distal cylindrical portion of the peduncle is formed the optic ganglion (Text-Fig. 3). This ganglion innervates the numerous ommatidia of the eye.

*The Occulomotor nerves* (Text-Fig. 1 om.n.) arise from the brain close behind the optic nerve and pass outwards along with it and supply the muscles of the optic peduncle.

*Antennulary nerves* (a.nl.) one pair. But actually it may be said to be two pairs. Each nerve is composed of two nerves running very close together and containing different kinds of fibres supplying the antennule and the antennary gland, the olfactory and tactile setae respectively. The nerve fibres actually arise from the middle region of the brain but the nerve leaves the brain at the anterior end inner to the oculomotor nerves.

*The tegumentary nerves* (t.n) which leave the brain at the posterolateral corner are a pair of nerves broader than all the other nerves starting from the brain, excepting the commissures. Each passes outwards and divides at a little distance from the place of origin into two branches which run together as one nerve for some distance and then separates, the anterior thinner branch supplying the integument at the rostral region, and the other branch innervating the anterior region of the whole integument.

*The antennary nerves* (a.n.2) are a pair of small nerves arising from the ventral side of the brain near the origin of the tegumentary nerves supplying the second antenna.

*The commissures* (com.) are a pair of large nerve strands arising from the posterior region of the brain. They pass posteriorly on the sides of the oesophagus and are connected with the ventral nerve mass. At each side of the oesophagus there is a ganglion, the *paraoesophageal ganglion* (p.g.) from which rises the *stomatogastric* nerve suplying the foregut. At the posterior region, where the commissures join the ventral nerve mass, the two commissures run close together and just anterior to this portion is the *post-oesophageal connective* (con.po) which is transverse connective connecting the two commissures. A small nerve starting from the portion immediately behind the paraoesophageal ganglion goes to the mandible.

The stomatogastric system of nerves (st.n.) supplying the region of the foregut is identical with that of *Cancer pagurus.*

*Ventral Nerve mass*: It is a circular nerve mass, with a foramen (fn) in the centre, through which passes the sternal artery from the heart, situated in the cephalothorax, just above the place where the median plate of the endophragmal skeleton ends. The nerve elements of all the post-oral cephalothoracic and the abdominal ganglia are contained in it. Five pairs of ganglia supplying nerves to the appendages are readily visible in the posterior region of the nerve mass. The abdominal ganglion is also distinguishable at the posterior end. The ganglia of the anterior region are difficult to be distinguished by the naked eye. However, they can be distinguished in stained preparations. The anterior region of the nerve mass, where the commissures from the brain meet, is narrow and the posterior portion is circular with the ganglia quite visible at the margin.

Five pairs of nerves, excluding the cardiac nerves, arise from the region between the starting point of the nerve to the cheliped and the commissure, as against four in *Cancer*. The first nerve (n. 1) sends branches to the mandible and the first and second maxillae. The other three nerves supply the three maxillipeds. All these four nerves start from the ventral nerve mass from the region in between the nerve going to the cheliped and the commissure and pass forwards and outwards one behind the other. In addition to these four pairs, there is another pair starting from the middle of this region, from in between the second and third nerves described above. This is the *recurrent cutaneous nerve* (r.c.n) described by Carlson (1905) and others. This nerve arises slightly dorsal to the plane from which the others start so that the four nerves and this nerve are not in the same plane. Each nerve passes upwards, forwards and outwards. It gives off a small branch, anteriorly at the region of the eye, which innervates the roof of the branchial chamber at the region behind the eye and the first spine of the anterolateral margin of the carapace. Then it passes horizontally beneath the integument of the carapace, towards the outer margin. At the region where it begins to pass horizontally it divides into two branches. The posterior one turns backwards and innervates the posterior portion of the integument. It turns round and sends branches to the region of the pericardium, pericardial pouch etc. The main branch gives off another branch anteriorly

which goes to the region behind the second and third marginal spines. It again divides into two, out of which the anterior main branch goes straight to the tip of the large lateral spine of the carapace giving off minute branches to the integument of this region. The other branch goes in between the other branches and innervates the region of the integument in between.

The fact that a nerve branch goes to the base of the lateral spine is conducive to think that it is sensitive. When the tip of this spine is touched with a hot needle the crab shows some wriggling movements, with the beating of the walking legs, especially the last peraeopod. At the same time, when the hot needle is brought at the tips of the other spines at the antero-lateral margin of the carapace, the crab shows no signs of sensitiveness. This shows that the lateral spines are slightly sensitive and hence the nerve supply.

The last five pairs of nerves are larger than the anterior ones and each pair goes to one peraeopod. The ganglia from which these nerves start are quite large and distinguishable at the sides of the ventral mass. After a little distance from their origin each divides into two and these two run close together.

*The abdominal nerve* (ab. n.) sarts from the posterior end of the ventral nerve mass. At this starting place there is a ganglionic swelling which represents all the abdominal ganglia fused together. The nerve passes posteriorly along the middle of the ventral side of the abdomen, sending off small branches to all the abdominal segments.

*The pericardial plexus and the cardiac nerves*

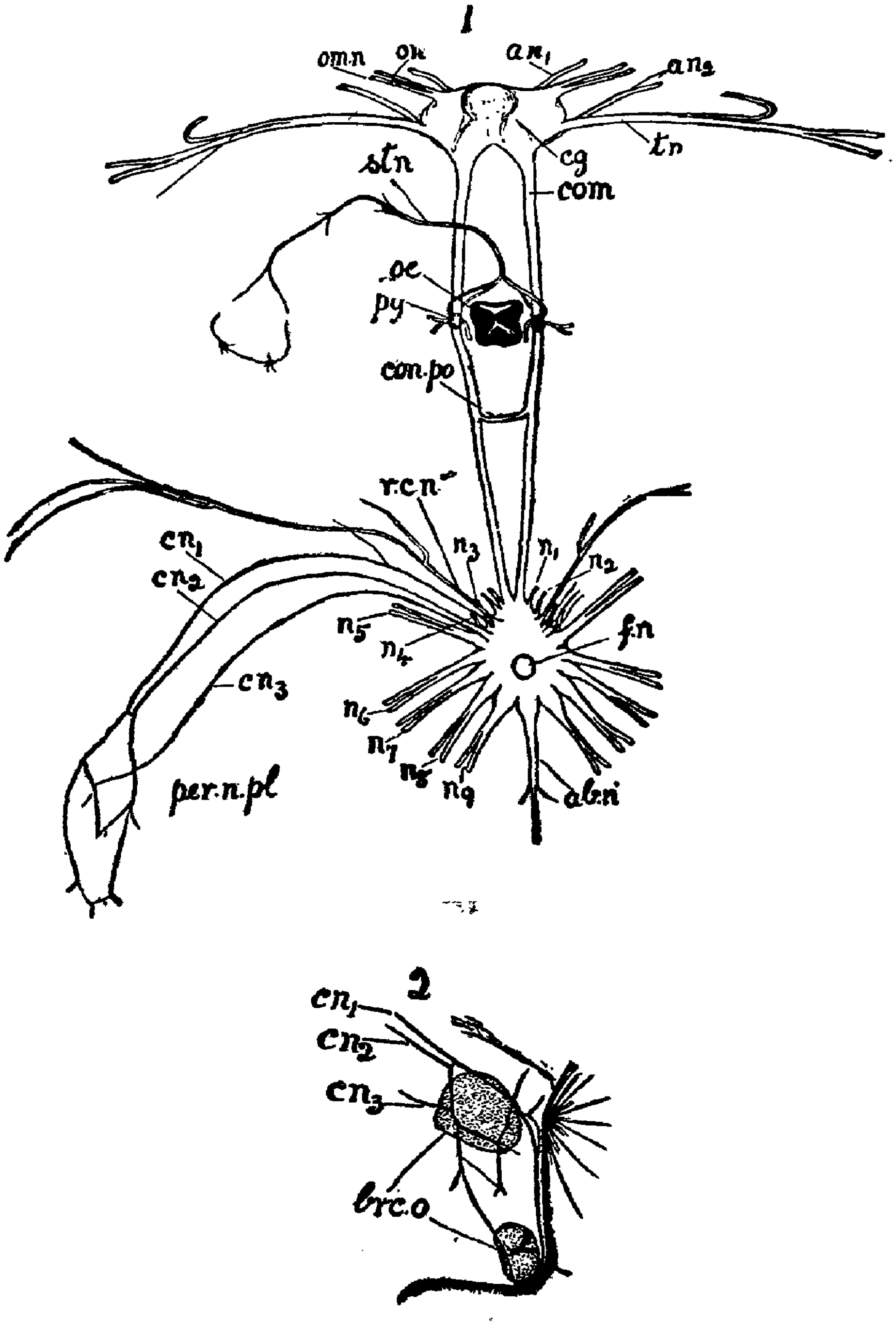
Pearson (1908) states that the cardiac nerves of Macrura do not appear to be present in the crabs. But three pairs of cardiac nerves are present in *Neptunus sanguinolentus* as recorded by Jolyet & Viallanes (1892) in *Carcinus maenas* and Connant and Clarke (1896) in *Callinectes sapidus.*

Lemoine (1868) describes in the crab a branch of the gastric nerve to the anterior and dorsal surface of the heart, which is directly connected with the cerebral ganglia and oesophageal commissures, but not with the thoracic ganglion. Plateau (1880) also agrees with them and they say that this nerve is accelerator in function. But Jolyet and Viallanes (1892) and Connant and Clarke (1896) failed to notice any trace of this nerve in the crabs *Carcinus maenas* and *Callinectes sapidus* respectively. They are of opinion

that both accelerator and inhibitory centres for the heart are situated in the thoracic ganglion and they have described a nerve plexus in the pericardial lateral walls and three pairs of nerves from there connected to the thoracic ganglion. According to Connant and Clarke the anterior pair of these is inhibitory and the posterior two pairs accelerator. Carlson (1905) confirms this opinion of Jolyet and Viallanes and Connant and Clarke. Police (1908) described in *Maia* three pairs of nerves approaching the heart from the sides. He also recorded the presence of the 'nerve of Lemoine' and called this nerve "nervus arteriosus medianus," but failed to notice its stout branches to the heart as decribed by Lemoine and others. Alexandrowicz (1932) also agrees with Police. But he calls it "nervus cardiacus anterior." According to them this nerve starts from the stomatogastric system, runs alongside the ophthalmic artery and penetrates the wall of the same, near its origin. Alexandrowicz describes a nerve going to the dorsal surface of the heart from the sides, which he calls "nervi cardiaci dorsales", as the main nerve connecting the heart with the ganglion, although he is not definite about the origin of this nerve. He also describes numerous anastomoses on the sides of the epimera from which the single nerve enters the dorsal surface of the heart.

The pericardial nerve plexus, the three pairs of cardiac nerves and one nerve going to the dorsal side of the heart as described by Alexandrowicz are met with in *Neptunus sanguinolentus*. On the lateral walls of the pericardium, inner to the openings of the branchio-cardiac veins, there are a number of nerves forming a net work (Text-Fig. 2). There are three pairs of very thin cardiac nerves (cn 1-3) arising from the dorsal side of the thoracic ganglion. The anterior one starts just behind the starting point of the recurrent cutaneous nerve. It runs for a little distance almost touching the latter, then separates and goes upwards along the inner edge of the endopleurite of the second thoracic arthrophragm to join with the second cardiac nerve, anterior to the opening of the first branchio-cardiac vein. The second cardiac nerve arises from the dorsal region of the thoracic ganglion, posterior to the first one, near the starting point of the nerve to the third maxilliped. It passes upwards along the edge of endosternite of the third thoracic arthrophragm and meets the anterior cardiac nerve as mentioned before. The third nerve (posterior) is the thinnest of the three and it starts near the starting point of the nerve to the cheliped. From there it passes sideways and then upwards and

joins a nerve branch running posteriorly and ventrally at the region of the opening of the first branchio-cardiac vein.




TEXT-FIGS. 1-2.

1. Dissection of the nervous system from the dorsal side; 2. Internal face of the right side of the pericardial lateral wall showing the pericardial nerve plexus.

The pericardial nerve plexus (Text-Figs. 1 & 2) is formed by nerve fibres running posteriorly, forming a net work, from the meeting place of the three cardiac nerves. At the meeting place of the first two cardiac nerves Jolyet and Viallanes (1892) have

found a ganglionic swelling. But in *Neptunus sanguinolentus* there is no such swelling. From the cardiac nerve junction two thin nerve fibres diverge out and pass backwards, near the dorsal and ventral borders of the opening of the second branchiocardiac vein.

At the region of the middle of the opening the dorsal branch gives rise to a branch which passes dorsally and is lost in the triangular plate at the inner corner of the fused thoracic epimera. This branch innervates the muscles on the above plate. Just posterior to this branch there is another thin nerve which passes slightly upwards and forwards and then enters the dorsal antero-lateral corner of the heart. It enters the heart along with the fibres of the dorsal anterolateral suspensary ligaments of the heart, not at the middle dorsal region of the heart as described by Alexandrowicz (1932). The dorsal branch starting from the meeting place of the first two cardiac nerves gives rise to a ventral branch at the region of its dorsal branch. This branch passes downwards and backwards and meets a similar branch given off dorsally from the ventral diverging branch from the meeting place of the first two cardiac nerves. From the meeting place of these, two minute branches are given off posteriorly. These diverge out and get lost in the tissues. One of them joins with a branch given off from the ventral nerve.

The ventral nerve passing along the ventral border of the second branchial cardiac vein meets the third cardiac nerve at about the middle of the ventral border of the opening. Then it gives off a branch upwards and backwards as mentioned above. It then passes further backwards and gives off a minute branch ventrally which is lost in the muscles.

The two nerves (dorsal and ventral) on their route pass one above and the other below the posterior branchiocardiac orifice. Posterior to this opening the two meet by a transverse nerve fibre and then anastamose giving off three branches to the extremity of the posterolateral corner of the pericardium where they get lost, supplying the muscles.

*Sense Organs.*

*The Eye*: The eye is situated at the distal extremity of a long movable optic peduncle contained, as usual, inside the orbit. The optic peduncle consists of two parts (1) A long narrow inner por-

tion which cannot be seen from the exterior with the narrower proximal end extending inwards in a slanting position towards the middle where it meets the similar part of the optic peduncle of the other side. (2) A broader cylindrical distal, externally visible portion, at the free end of which is the hemispherical visual organ. The whole of the optic peduncle, excepting distal black region and the portion where the two parts of the peduncle meets, is covered with a thick calcified cuticle. Thus the two parts are separated by a region of soft chitin, so that a forward and slightly upward and a backward and downward movements are possible These movements are caused by two muscles inside the peduncle, a ventral flexor and a dorsal extensor which is the bigger of the two.

At the distal end of the cylindrical region is the black hemispherical visual region covered by the cornea, which is invaded by only one tubercle on the dorsal surface, as against two described by Pearson. This tubercle is not black and is narrow and extends upto three fourth of the length of the black region. The cornea is a thin layer of chitin which is continuous with the thick strong calcified cuticula of the remainder of the peduncle. Beneath the cuticle is present the usual inner layer of epidermis, resting on a basement membrane. But in the visual region this cellular layer is very thick and is called *ommateum* which rests on the basement membrane and are continuous with the corresponding parts in the proximal portion of the peduncle.

The *corneal cuticula* as in other Decapods, show a faceted nature, microscopically. It is divided into an immense number of small hexagonal facets. But all the facets are not exactly hexagonal. Some at the region of the edge of the cornea and the region near the tubercle show a tendency towards tetragonal shape (Text-Figs. 4 & 5).

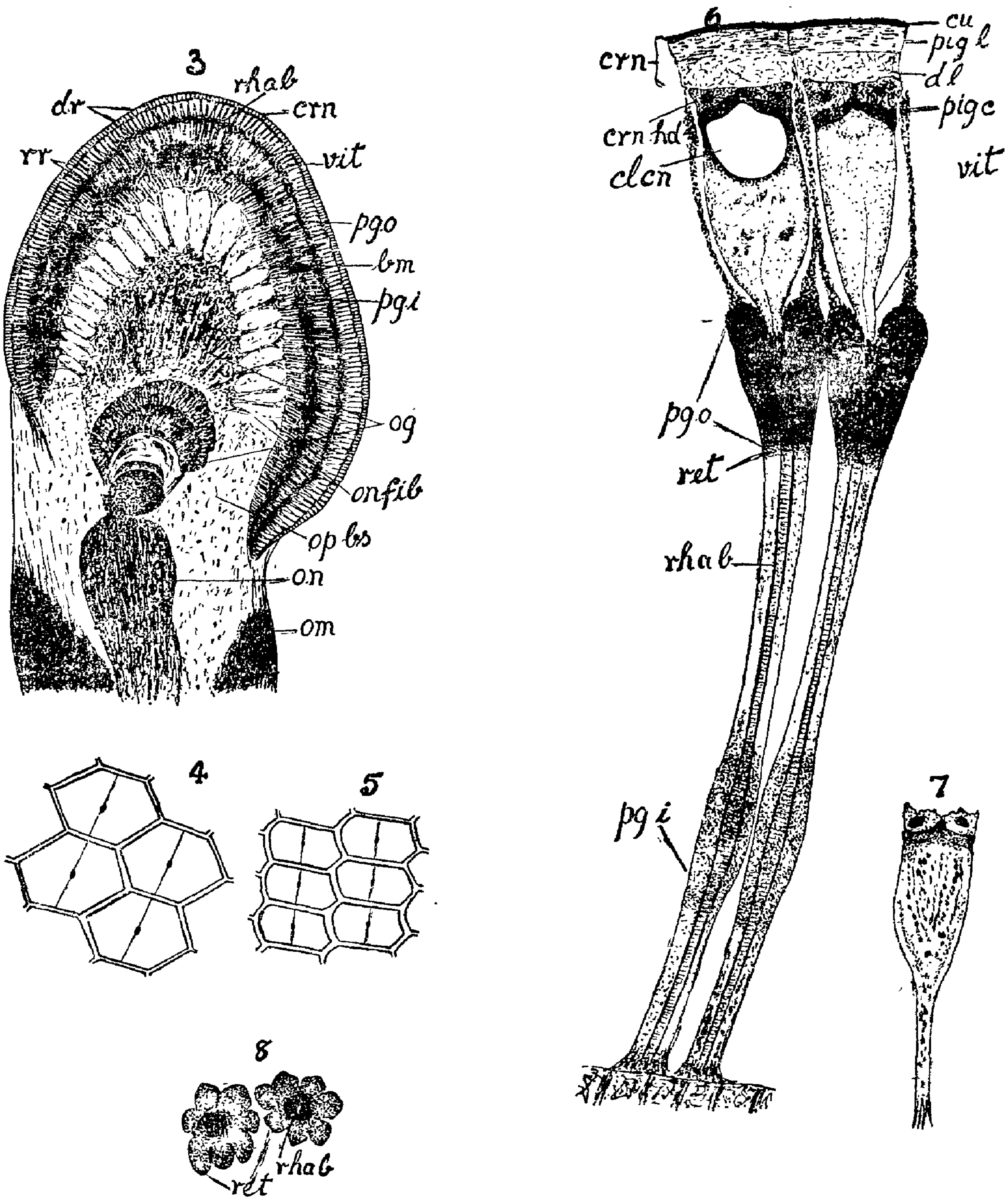
The hexagonal arrangement is characteristic in all Crustaceans except the Decapods, according to Parker (1891). In Decapods the arrangement is either hexagonal or tatragonal. He states further that "in the Brachyura as well as in three families of the Macrura, the Hippidae, the Paguridae and the Thalassinidae, the arrangement of the ommatidia is invariably hexagonal. In the remaining Macrurous Decapods with a few exceptions in *Galathes* and *Typton*, the ommatidia are grouped on the tetragonal plan." He supports, this statement with the reference to a series of literature.

According to Parker (1890) in the corneal cuticula of the eye of the lobster, *Homarus americanus,* although as a rule the outline of the facet is square, hexagonal facets are present at the margin of the retinal area, close to where the ommateum passes over into the undifferenciated hypodermis. He has also recorded it from other specimens like *Palinurus, Scyllarus* etc. From this he concludes that the hexagonal arrangement is phylogenetically more primitive than the tetragonal arrangement and for the phenomena of both arrangements seen in the same specimen he gives the explanation that the hexagonal arrangement in the early stages is replaced by the tetragonal in the adult. He explains the presence of hexagonal facets towards the periphery of the cornea of *Astacus, Homarus* etc., with the same argument. But in the cornea of *Neptunus sanguinolentus* the facets in the middle and other regions are invariably hexagonal. But some facets near the periphery are elongated and more or less rectangular with the two sides of the hexagon much elongated and the other four sides short and the angle between the shorter sides rounded up (Text-Figs. 4& 5) thus forming more less a rectangle. Parker's explanation does not seem to apply here. The occurrence of rectangular facets near the periphery may probably be due to growth of the region and the surrounding regions.

Each corneal facet is bisected by a fine straight line running from the middle of one side to that of the other side. There is a small round point in the centre. In a crab with a carapace breadth of 8 cm. across, the length of a hexagonal corneal facet in between the parallel sides are $43 \cdot 8\mu$, $45 \cdot 4\mu$ and $48 \cdot 6\mu$ respectively. The distance between respective corners diagonally are 55·1, 51·8 and $50 \cdot 2\mu$ respectively. The cornea has the three layered structure.

On the inner side of the cornea the ommateum is divided into several elements, each placed beneath a corneal facet, so that these parts have the arrangement as the corneal facets. Each element is an *ommatidium,* which extends from the corneal facet to the basement membrane inside. The number of ommatidia equals the number of corneal facets.

*Structure of an ommatidium* (Text-Fig. 6): Each ommatidium consists of a cluster of cells which are regularly arranged about a central axis. As in other Decapoda, immediately beneath the corneal cuticular facet is situated a pair of flattened triangular cells. These are the *cornaegen cells* (corneal hypodermis of Parker) (crn.).




TEXT-FIGS. 3-8.

3. Longitudinal section through the eye; 4. Surface view of the cornea at the central portion of the eye showing the facets; 5. Surface view of the cornea at the peripherial region of the eye; 6. Section of two Ommatidia; 7. Cone cell showing the separation of the four cells at the proximal region; 8. Transverse section through two Ommatidia at the region of the rhabdome and retinulae

Each cell is taller at the edges and shorter towards the middle region so that it is more or less triangular, and the middle portions of the cone cells, which lie beneath project forwards. Each cell, has a maximum breadth of 19 to 20μ and maximum length of 21μ in the crab mentioned above.

Beneath the corneagen cells lie the *cone cells* or *vitrillae* (vit). There are four tall cone cells in each ommatidium. Each cell is 155μ in length at the sides, the middle region being longer because of the shape of the cornaegen cells, as in *Homarus americanus and Palaemonetes,* although these pointed ends do not touch the corneal cuticle as in those cases. The distal ends of the cone cells are in touch with the proximal ends of the cornaegen cells. Each cell has a nucleus near the distal extremity, just below the hypodermal cells. Below the region where the nuclei are situated, the four cells enclose the *crystalline cone* (cl. cn.) which is secreted by the cone cells. Consequently in a longitudinal section of the eye, the crystalline cone of different sizes, varying from the smallest size (sometimes even absent in some ommatidia) to the biggest, covering the whole anterior wider region of the four vitrillae, can be seen. The pigment cells are present on the sides of the vitrillae, though they are not so defined as in *Cancer.* Their continuations which pass along the sides of the cone cells leaving intercelluar spaces between them are in connection with the distal ends of the retinulae surrounding the rhabdome. The proximal ends of the cone cells are very narrow, the breadth of the proximal region of the four vitrillae of an ommatidium being less than one fourth of the breadth at the distal region. At the distal end the four cells are colsely applied to each other. At the proximal narrow region they can be traced to a region immediately distal to the distal end of the rhabdome. Schultze (1868) in *Astacus* and Parker (1891) in *Homarus* and *Palinurus* have demonstrated that the proximal fibrous ends of the four cone cells separate, extend proximally and terminate at the basement membrane. In *Neptunus sanguinolentus* the four fibrous proximal ends were traced near the distal end of the rhabdome (Text-Fig. 7). Due to the intense pigment at the distal end of the retinulae it is not able to trace their terminations.

The region of the ommatidium extending from the corneal facet upto the inner ends of the cone cells is known as the *dioptical region.* (Text-Fig. 3).

Beneath the vitrillae is situated the rhabdome (rhab) which is rod-like and transversely striated. The rhabdome is slightly

broader at the region three fourth of the length from the distal end, being 5μ. The other portions are 4·2μ wide. The rhabdome is completely covered by the retinulae (ret.). There are seven retinular cells (Fig. 8) and those extend from the proximal end of the vitrillae to the inner basement membrane. The nuclei of these cells are situated at the distal extremities which are rounded and wider. All the distal extermities of the seven cells completely surround the rhabdome and the pigments at this region are so much concentrated as to hide the latter. This concentration of the pigments of all the ommatidia gives rise to a well marked pigment band (pg.o). At about three fourth length from the distal end of the retinulae there is another concentration of pigments around the rhabdome. The pigments are not so concentrated as in the other region, so that the rhabdome can be made out in the middle. At this portion the rhabdome itself is slightly broader as mentioned above. This concentration of pigment also forms a continuous pigment band (pg. i), though not so dark as the other, inner to the well marked inner pigment band of Pearson.

The rhabdome and the retinulae together form the *retinal part* of the ommatidium. The fibres of the optic nerve perforate the basement membrane and enters the retinulae, so that this retinal region is the real part, sensitive to light.

Thus each ommatidium is a row of cells placed end to end to form a tubular structure enclosed within a layer of pigment, which separates the different ommatidia from each other. So the retinulae of each ommatidium receive light only through the outer corneal facet.

The total length of an ommatidium, without including the cornea, in the above crab is 0·5 to 0·51 mm. The breadth of the ommatidium at the different regions is as follows:—

| | |
|---|---|
| At the region of the cornaegen cells . | 42·11μ |
| At the region of the middle of the vitrillae . | 36 to 42μ |
| At the region of the distal end of the retinular cells, i.e., outer pigment band . | 29 to 32μ |
| At the region of the inner pigment band .. | 16·2μ |
| At the region just below the above region .. | 19·5 to 22·5μ |

*The Otocyst.*

The otocyst is situated in the enlarged basal segment of the antennule. It is a chitinous sac suspended from the dorsal wall of that segment in which a transverse line can be noted externally representing the place to which it is attached. In adult crabs the sac is completely closed and there are no otoliths inside. The average of a number of measurements of the otocysts in specimens approximating 7 cm. across the carapace (without including the lateral spines) give the following results:—

| | | |
|---|---|---|
| Greatest length | .. | 2·7 mm |
| Greatest breadth | .. | 2·5 mm |
| Greatest depth | .. | 1·3 mm |

The sac is irregular in shape, caused by three invagintions of the wall projecting into the lumen and forming sensory cushions.

Three kinds of hairs are situated on the walls of the otocyst, projecting into the lumen. Hensen (1863) divides them into the following classes (i) Hook hairs (Hakenhaare); (ii) Thread hairs (Fadenhaare) and (iii) Grouped hairs (Gruppenhaare).

The hooked setae are found on the posterior convex portion of the wall of the otocyst. Their shafts are bent and small, averaging 45μ in length and 3 to 4μ in diameter. These hairs are not fringed with lateral hairs. The *thread hairs* are the largest of the bristles in the otocyst. There are 34 of them arranged in two or three rows on the anterior region of the floor. Each hair is about 325μ in length. But the diameter differs from that of *Carcinus maenas* described by Prentiss (1901). At the base there is a swollen portion which is 16μ wide and the rest of the hair is 10μ in diameter. There are no barbs on the shaft of the hair. The *group hairs* are blunt, barbless hairs about 120μ in length and 6μ in diameter situated on the lateral wall of the sac. All the setae are innervated from the otocyst nerve.

Different types of sensory setae are met with in *Neptunus sanguinolentus* as in other Decapods. Tactile setae are present on various parts of the body. Most of the appendages have sensory setae on them especially the antennules and the antennae and the appendages of the mouth part. Structure is as usual.

Olfactory setae are found in the two antennae. Those on the endopodites of the second antenna and the antennule possess a

transverse suture in the middle as described by Pearson. The number of setae on each ring varies from 1 to 4. On the backside of the exopodite of the antennule the setae are very small and bristle-like and there is only one such seta on each ring of the tip portion. Gustatory setae, which are modified olfactory setae, are present in the appendages of the mouth parts.

## EXPLANATION OF TEXT-FIGURES

abn—Abdominal nerve; an1—Antennulary nerve; an2—Antennary nerve; bm—Basement membrane; brc.o—Openings of branchio cardiac veins; cg—Cerebral ganglion; cl.cn—Crystalline cone; cn 1-3—Cardiac nerves 1 to 3; com—Commissure; con.po—Post Oesophageal connective; crn—Cornea; corn.hd—Corneal hypodermis; cu—Cuticle; dl—Deep layer of the cornea; dr—Dioptical region; fn—Foramen of the ventral nerve mass; n 1-9—Nerves from the ventral mass; oe—Oesophagus; og—Optic ganglion; om—Ocular muscles; om.n—Oculomotor nerve; on—Optic nerve; on. fib—Optic nerve fibres; op.bs—Ophthalmic blood sinus; per.n.pl—Pericardial nerve plexus; pg.—Paroesophageal ganglion; pg.i—Inner pigment band of the eye; pg.o—Outer pigment band of the eye; pig.c—Pigment cell; pig.l—Pigment layer; r.c.n.—Recurrent cutaneous nerve; ret—Retinulae; rhab—Rhabdome; rr—Retinal region; stn—Stomatogastric nerve; tn—Tegumentary nerve; vit—Vitrillae.

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# The Anatomy of the Crab *Neptunus Sanguinolentus* (Herbst)

## PART III: Respiratory, Excretory and Blood Vascular Systems*

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### *Introduction*

The present contribution formed part of a study of the complete anatomy of the edible crab *Neptunus sanguinolentus* (the generic name changed to *Portunus* recently) in comparison with the European form *Cancer pagurus* (Pearson, 1908), emphasising on the points which differ and is in continuation of the two previous parts, first dealing with the internal skeleton and musculature and the second on the nervous system and sense organs.

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### *Respiratory System*

As early as 1839 Milne Edwards described the respiration in Crustacea Decapoda. The movement of the scaphognathite aiding in respiration was first observed by him. De Haan (1850) and Garstang (1896) studied respiration and recorded the occurrence of the reversal of the respiratory current in some crabs. Bohn (1897, 1898 & 1902) is of opinion that the reversal of respiratory current is of universal occurrence in Brachyura. Lim (1917) has studied respiration of *Carcinus maenas* and he confirms Bohn's opinion. Smith (1942) also studied the respiratory system and the structure of the branchiae.

* *Edited from a thesis approved for the M.Sc. degree.*

The respiratory organs and their arrangements in *Neptunus sanguinolentus* are essentially the same as in *Cancer pagurus*. The differences are noted below.

In the branchial chamber there is no well defined ridge in the anterior region of the floor and because of the absence of this branchial ridge the entrance between the branchial and pre-branchial chambers is not so narrow as in *Cancer*. Unlike in *Carcinus maenas* described by Lim (1917) the branchial and pre-branchial chamber is in communication with the exterior by the two inhalant apertures and one exhalant aperture.

The *anterior inhalant aperture* is a well defined, large and transverse opening leading into the anterior region of the branchial chamber, situated in front of the coxopodite of the chela. Water entering through this aperture is strained by the thick growth of setae found on the ventral region of the branchiostegite, in front of the aperture on the posterior borders of the coxopodite and flabellum of the third maxilliped and on the dorsal surface of the coxa, basiischium and meropodite of the cheliped. The *posterior inhalant aperture* is a very narrow transverse slit beneath the postero-lateral corner of the carapace and above that coxopodite of the last peraeopod. This aperture is not at all visible, except when the corner of the carapace is slightly raised. This aperture does not seem to allow much water to enter the branchial chamber. The *exhalant apertures* are wide openings on both sides of the mouth, leading from the branchial chambers through the pre-branchial chambers.

*The gills* (Text-figs. 1 & 2): The gills are situated inside the branchial chamber on the dorsal side of the thoracic epimera on both sides of the thorax. The three usual types of gills, i.e., the podobranch, the arthrobranch and the pleurobranch are present. Altogther there are 8 gills on each side in *Neptunus sanguinolentus* as against 9 in *Cancer pagurus*, *Carcinus maenas* etc., the podobranch on the third maxilliped being rudimentary in the former. In *Callinectes sapidus* also Cochran (1935) has recorded 8 branchiae only on each side.

As the gills are found on the epimeral extension and arthrodial membrane without any well-marked separation it is difficult to group the gills into arthro and pleurobranchs.

*First gill* (Text-Fig. 1·gl): is the podobranch of the second thoracic somite and it arises from the coxopodite of the second

maxilliped. Its inner face is closely applied to the basal portions of the second, third and part of the fourth gills, so that its apex points backwards and outwards, while al the other gills are placed in a transverse direction inside the body. In a crab measuring 9·5 cm. across the carapace (without including the lateral spines) the length of the first gill is 11 mm.

*Second gill* (g2) : This is the arthrobranch of the second thoracic somite and it is longer than the first gill, being 16·5 mm. in the same crab. Its axis is more or less at right angles to that of the first gill and it lies upon the basal portion of the fused epimera of the first and second thoracic somites and the inner anterior portion of the third thoracic epimeron.

*Third gill* (g3): The third gill of *Neptunus sanguinolentus* corresponds to the fourth gill of *Cancer pagurus.* The podobranch of the third maxilliped which is the third gill in *Cancer,* is only present, in *Neptunus,* as a rudimentary protuberance, with the tip pointed and covered with setae, directed vertically in between the bases of the third and fourth gills. The third gill in *Neptunus* is the anterior arthrobranch of the third thoracic somite and it arises together with the *fourth gill* (g4) from the arthrodial membrane of this somite. These two gills lie on the third thoracic epimera. The third gill is 23 mm. and the fourth one 28 mm. in length. The third gill of *Cancer* being absent here, the fourth gill does not possess a notch at its base.

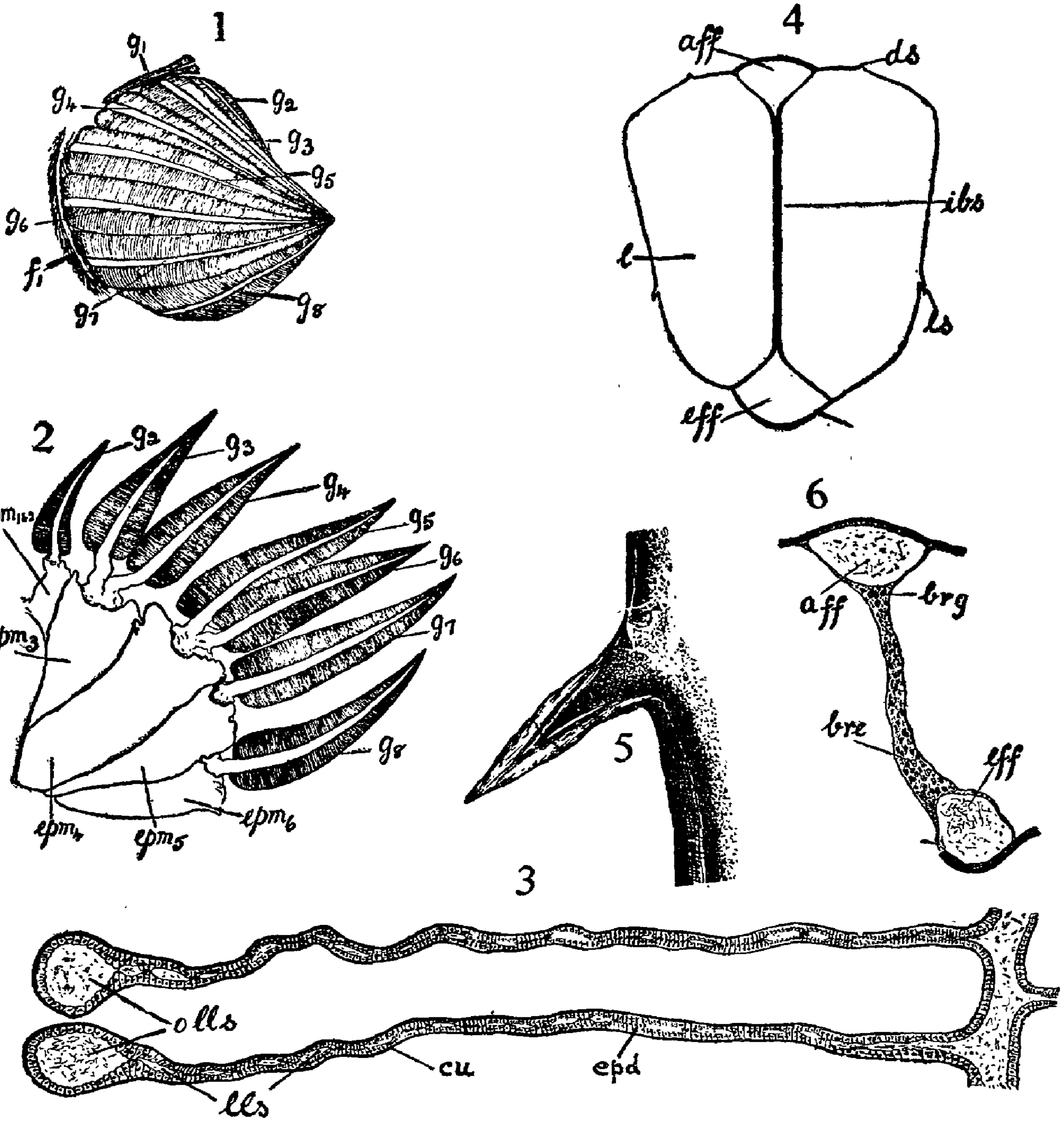
*The fifth and sixth gills* (g5, g6) are the same as the sixth and seventh gills of Cancer. They measure 30 mm. and 31 mm. respectively.

*Seventh gill* (g7) is the pleurobranch of the fifth thoracic somite and arises from the outer anterior portion of the fifth thoracic epimeron. It measures 29 mm. in length.

*Eighth gill* (g8) arises from the outer anterior portion of the sixth thoracic epimeron, upon which it lies. Length is 28 mm.

*Structure of the gill* (Text-Fig. 3) : Each gill is phyllobranchiate type and triangular in cross section, with the afferent branchial blood vessel running along the middle of the dorsal side and the efferent branchial vessel on the ventral side. Setae with minute plumes, here and there, are present on the walls of the efferent branchial vessels. Excepting the first gill in which the apex is pointed backwards, all the other gills have their apices pointing in-

wards. The branchial septum is present stretching in between the afferent and efferent vessels.




**Text-Figs. 1-6.**

1. Dorsal view of the gills of the left side; 2. Gills of the right side stretched showing their attachment; 3. Section through the gill lamellae; 4. Diagramatic transverse section of the gill; 5. Lateral spine on the gill lamella; 6. Transverse section through the gill showing the branchial septum.

The gill is composed of numerous lamellae, placed one behind the other. All these lamellae are covered with a layer of chitin, which is continuous on the outside of the afferent and efferent ves-

sels also. The chitin being 0·005 to 0·007 mm. in thickness is permeable to gases, water and salts. All the lamellae possess the lamellar sinuses, outer lamellar sinuses, epidermal cells etc., as in *Cancer*.

On each gill lamella there are some spinous (Text-Fig. 4) projections on the outer chitinous covering. There is a pair of the spines on the lateral side (l.s.) one on each side at about three-fourth the length from the dorsal side. This spine is directed downwards. The spines of all the lamellae together form continuous lines on the lateral sides of the gills. Each spine (Text-Fig. 5) is simply a projection covered by chitin and is divided into two segments by a transverse line about three-fourth the length from the proximal end. The distal segment is pointed distally.

Another pair of spinous projections is also present on the dorsal side of each lamellae, one on each side of the afferent branchial vessel (Text-Figs. 4, d.s.). These are smaller than the spines on the lateral sides and are only simple, pointed projections directed upwards. These spines also form a continuous line on the dorsal side, one on each side of the afferent branchial vessel. Another pair of very small spinous projections are present a little below the lateral spines. These are very small projections from shallow depressions on the chitin, so that only the very tips are seen outside. However, these also form a very faint continuous line on the lateral side of the gill. The projections belonging to the third category are sometimes present also on some other places of the walls of some of the gills.

The function of these spines are very doubtful. The lines formed by the lateral spines of two gills will be touching each other when situated side by side, and this line is formed by downwardly directed spines. So these spines may probably prevent sand particles, other impurities and external parasites coming along with the respiratory current of water, from reaching above this line to the dorsal side. An examination of the gills and the gill chamber will make it more clear. Most of the gills of *Neptunus sanguinolentus* are infected by a cirripede commensal, *Dichelapis transversa*. But usually most of these parasites are found on the ventral side of the gill below the lateral line formed by the lateral spines of the gill lamellae. The number of cirripedes found on the dorsal side are very few when compared with the ventral side. So the lateral spines may prevent the cypris larvae of these commensals from getting above the lateral line, to a large extent. Also the floor of

the branchial chamber is always full of sand particles and other impurities. The spines may also prevent them going up along with the respiratory current, acting as a sieve.

The dorsal line of small spinous projections may be helpful in cleaning the setae of the flabella of the maxillipeds. These flabella are used for cleaning the branchiae and the branchial chamber. Some of the impurities may be sticking on to the setae of these. So when these flabella are moved sideways, very close to the dorsal surface of the gills, the setae will be touching the ends of the spinous projections and this may help in the removal of particles from the setae.

Scattered cells constituting the branchial excretory organ are seen in the branchial septum. According to Cuenot these cells are excretory in function.

Allen (1892) has described in *Palaemonetes varians,* two types of branchial glands in the gill, near the blood vessels. Cuenot (1895) has recorded such glands close to the efferent vessels in several of the Brachyura. But Pearson has failed to notice them in *Cancer pagurus.* In *Neptunus sanguinolentus,* however, there are a few glands, belonging to both types of glands described by Allen, near the efferent branchial vessel (Text-Fig. 5).

*Respiratory Current and its Reversal* :

When a specimen of *Neptunus sanguinolentus* is held with the exhalant apertures above the water surface, a continuous current of water can be seen flowing out through the exhalant orifices on the sides of the mouth. This current of water enters through the inhalant aperture below the base of the cheliped, passes through the branchial and pre-branchial chambers and goes out through the exhalant apertures. If a crab is watched carefully from the front side, through the exhalant apertures can be noticed the anterior end of the scaphognathite moving up and down. This undulating movement of the scaphognathite, which was first noticed by Milne Edwards (1839) is considered by many authors as the cause of the expulsion of water and thus causing a steady current flowing through the exhalant apertures. The rate of movement of the scaphognathite can be noted with the help of a stop watch.

*Rate of respiration* : In a female specimen at rest (measuring 9·5 cm. across the carapace) the rate of movement of scaphognathite was 130 times per minute. A small female crab with a

carapace breadth of only 3·5 cm. and infected by a parasite *Heterosaccus ruginosus* (George, 1959), showed a very rapid movement of scaphognathite, about 200 times per minute, difficult to be counted exactly. This rapid movement may be due to the crab being young and consequent higher rate of metabolism or due to parasitisation. But parasitation does not seem to affect the respiraory movement, because another male crab measuring 8 cm. across the carapace and infected by a parasite had the scaphognathite moving at the rate of 125 times per minute. So the increased rate of movement in the former crab is probably due to the crab being young and thus needing more aeration of the system due to increased metabolism.

*Reversal of Current:* At frequent intervals a change in the direction of the normal current was noticed, during which the water enters through the exhalant apertures and comes out through the inhalant apertures. Though this reversal of respiratory current was observed by Garstang (1896) in *Corystes cassivelaunus, Portunus nasutus* etc., it has not been recorded by Pearson (1908). According to Bohn (1902) this is of universal occurrence in Brachyura.

The reversal of the current can be observed unaided especially when there are suspended impurities. However, it was studied with the help of neutral red as described by Garstang. The crab was placed in a glass trough with sea water just enough to cover the body of the crab, so that the currents of water emerging through the exhalant apertures can be clearly seen, close to the surface. Water coloured with neutral red was then added by means of a pipette to the region in front of the base of the chelipeds. This coloured water was drawn in through the inhalent apertures and came out through the exhalant apertures. This is the normal respiratory current. But more rarely the coloured water was sucked in through the exhalant apertures on both sides and were seen to be sent out through the inhalant apertures. Suddenly again the normal current was set up.

According to Bohn (1902) the reversal takes place in order to rest the fatigued muscles of the scaphognathite. The author failed to observe any pause in the movement of the scaphognathite, for the reversal of current. It appears to be due to a reversal of the direction of the movement of the *scaphognathite*. It is probable that this reversal of movement helps to wash out particles

which accumulate and clog the gill surface during the prolonged flow of outside water into the chamber. This conclusion gains strength when the crab is observed carefully at the moment of reversal. The first indication of reversal is usually a cloud of dust particles emerging from the aperture, into which water was flowing hitherto. The particles of suspended impurities continue to come out of the inhalant aperture, as long as the direction is reversed. After a short interval of a few seconds the normal direction of flow is resumed. During these long periods when water enters through the inhalant apertures the water does not show any trace of particles. Hence it is inferred that suspended impurities become stuck to the gills, during the period of normal flow and the brief reversal of current is likely to dislodge these particles from the surface of the gill, especially the ventral surface. Lim (1917) says that "the reversal of the direction of the respiratory current is more frequent when the crab is buried or when a strong ink suspension is used to show the direction of the respiratory current,' thereby indicating that there is a relation between the amount of suspended particles and reversal of respiratory current.

### *Excretory System*

The excretory organs in Crustacea decapoda was studied by Marchal (1892). Cuenot (1895) studied excretion in Crustacea by injection of colouring matters and detected the presence of the branchial excretory organs. Waite (1899) described the structure and development of antennal glands in the lobster *Homarus vulgaris.* The antennal glands of Decapods was the subject of a study by Schlieper and Herrmann (1930). Nancy Samuel (1945) studied the antennary glands and their excretions of three South Indian Decapods, namely *Panulirus polyphagus,* the amphibious crab *Paratelphusa guerini*, and the sea crab *Scylla* sp:. She tested the urine of the three species for sodium chloride, urea, ammonia, uric acid, creatinine, guanin etc., and has given a comparative account of the antennary glands of all the three species. The excretory and osmoregulatory functions of the glands are discussed, and according to her "the antennary glands are the primary osmoregulators in Decapod crustacea."

Different organs appear to be concerned with excretion as met with in *Cancer,* namely, the antennary glands, the secretory cells of the digestive gland, and the branchial excretory organ. All of them are more or less like that of *Cancer.*

*Antennary glands*: (Text-Figs. 7, 8,) The whole organ consists of (a) the glandular mass, made up of the labyrinth and the end sac, (b) the bladder with its extensions and (c) the ureter which opens to the inside beneath the operculum. Unlike in *Cancer-pagurus, Paratelphusa* and others the glandular mass in *Neptunus sanguinolentus* consists of the laybrinth alone, the end sac being atrophied as in *Scylla sp.* (Nancy Samuel 1945). The gland is situated in a vertical position in cephalic region immediately behind the eye socket. It is triangular in shape and light green in colour. The apex of the triangle is towards the outer side. Posteriorly there is a depression in the middle region of this triangular gland so that the edges are widely ridged. In a crab measuring 10 cm. across the carapace the length from the apex to the base of the antennary gland is 5 mm and the length of the base is also about 5 mm.

The glandular mass (Text-Figs. 9 & 10) consists of a number of convoluted tubules which are not in separate blocks as in *Scylla sp.* The wall of each tubule is made up of a layer of columnar epithelial cells which are 10 to 17μ in length. The nuclei of these cells are 5 to 6·5 × 6 to 7μ. The space in between the tubules is filled with inter-tubular tissue (it. t.) in which cell boundaries are not clearly marked off. The lumen of the tubules are also filled with intratubular tissue (ft. c.) showing a honey comb appearance as in *Scylla sp*: Here also the cell boundaries are not clearly marked off. There is an outer cellular membrane (o.c.m.) with cells 15 to 20μ in length in the wall of the renal tube from which the tubules pass inwards as trabeculae.

The *bladder* is thin-walled and consists of a small main vesicle and several extensions or lobes. All the lobes present in *Cancer* are not present here. The main vesicle is situated above the antennary gland and its cavity is continuous with that of the renal tube at the outer and anterior end of the gland. In front of that it is connected with the ureter. It has (Text-Fig. 7) all the extensions of the lobules found in *Scylla* sp: The Mid-dorsal extension of the cerebral lobe is pouchlike and is in connection with its fellow on the other side by a narrow passage, unlike that of *Cancer*. The median ventral or oesophageal lobe is divided into two and situated on the two sides of the oesophagus. The posterior extensions starting from the inner dorsal corner of the vesicle, representing the epigastric lobe of *Cancer*, lie on the sides of the stomach. The mid-lateral extensions (paragastric lobe) lie on the

ventral side of the foregut. The anterolateral extensions are situated on the sides extending from the outer corner of the vesicle. In between the anterolateral and the mid-lateral extensions there is

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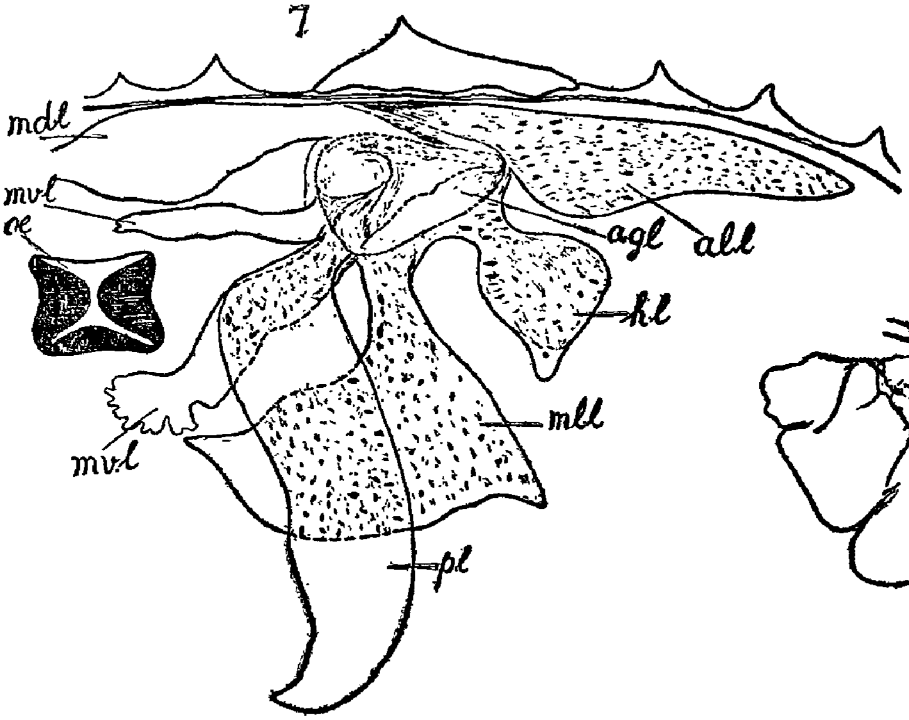


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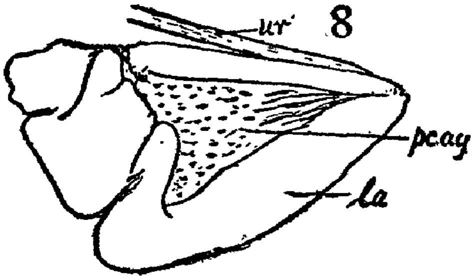


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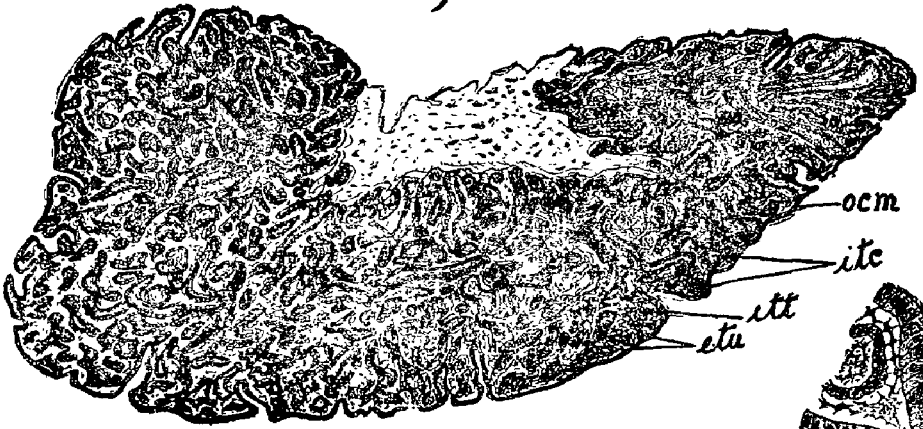


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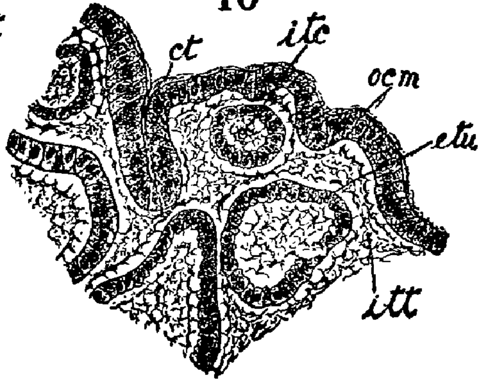



TEXT-FIGS. 7-10.

7. Dissection of the right side to show the extensions of the bladder from the dorsal side; 8. Posterior view of the antennary gland; 9. Vertical longitudinal or sagittal section through the antennary gland; 10. Portion of the renal tubules.

a small pouch-like lobe which is absent in *Scylla*, situated on the roof of the pre-branchial chamber. This represents the hepatic lobe of *Cancer*.

The ureter starts from the anterior outer corner of the main portion and leads inwards along the edge of the antennary gland. It runs obliquely along the side of the eye socket and leads forwards to open beneath the operculum, which is situated at the posterior and outer corner of the basal segment of the antenna, towards the ventral side. The operculum and the excretory orifice are the same as in *Cancer*.

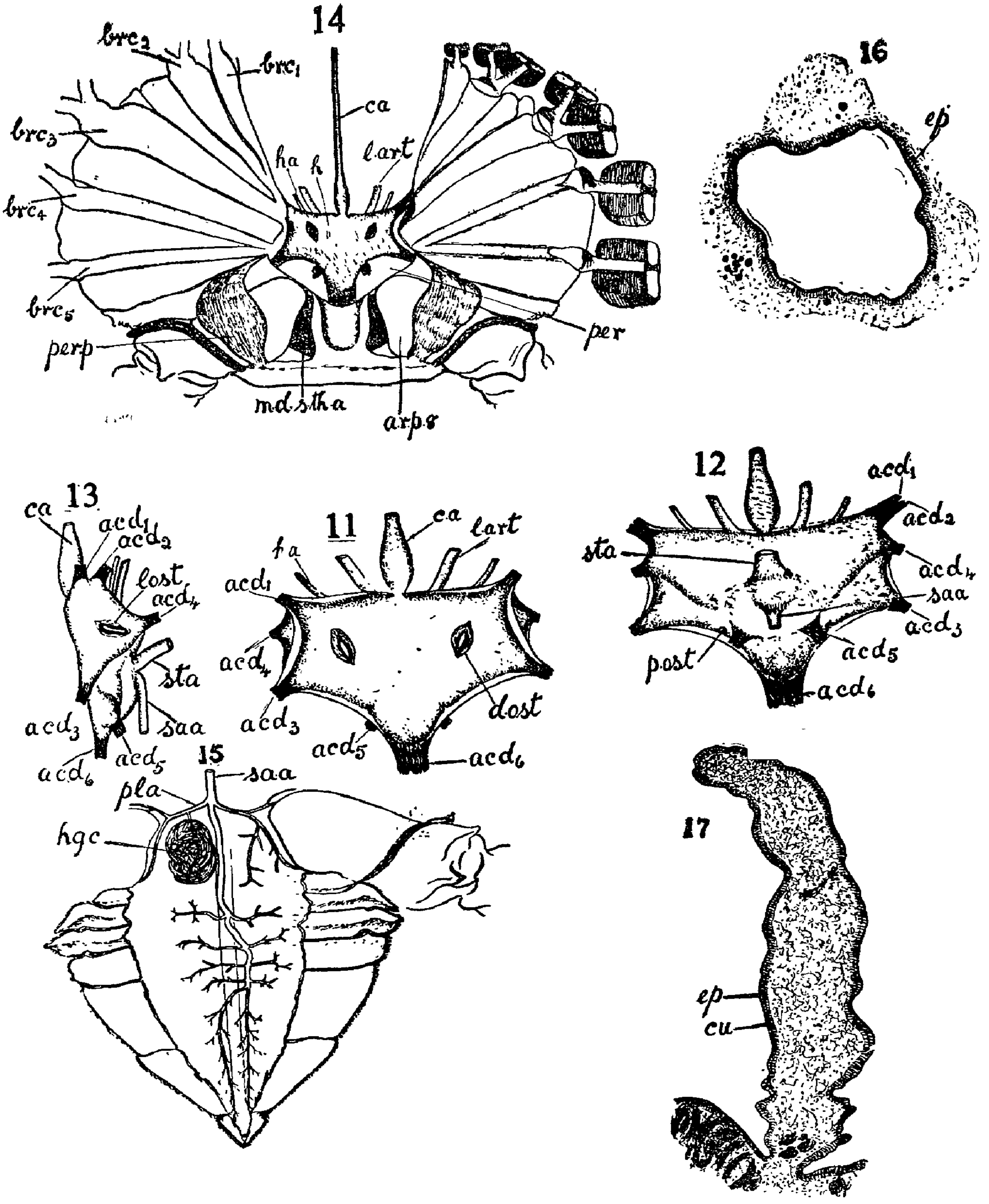
*Secretory cells of the digestive glands*: According to Cuénot (1895) the digestive gland in addition to the functions of digestion and absorption has also the function of excretion. Part of the contents of the ferment cells are of an excretory nature according to Pearson. Methylene blue injected into the body was detected in the ferment cells of the digestive tubules by them. In *Neptunus sanguinolentus* also methylene blue injected into the body was detected in the excretaory cells of the digestive tubules. However, Reddy (1937) agreeing with Yonge (1924) does not give much imporance to this function of the secretory cells.

*The branchial excretory organs*: The branchial excretory organ is represented by scattered cells found in the portion of the gills in between the afferent and efferent branchial vessels, (fig-6). These cells may play an important part in the transport of water and salts in osmoregulation.

### *Blood Vascular System*

In his "Histoire Naturelle de Crustaces", Milne Edwards (1834) described the blood vascular system of *Maia* along with other systems. Cuénot (1895, 1903) studied the vascular system and the glands connected with it. The haemoglobin content of the blood of Crustacea and the coagulation of blood were studied by Smith (1909) and Tait (1908, 1911 & 1918) respectively. Izquierdo (1931) has made intensive study of the Crustacean heart muscle and its innervation. Observations on the rate of heart beat of *Callinectes sapidus* were made by Weathersby (1936).

The scheme of circulation in *Neptunus sanguinolentus* is identical with that of *Cancer pagurus*. So only the differences in the course of the blood vessels, shape of heart etc., are mentioned here.




**Text-Figs. 11-17**

11. Dorsal view of heart; 12. Ventral view of heart; 13. Lateral view of heart; 14. Dissection of the posterior region of the thorax from the dorsal side to show the heart, pericardium, branchiocardiac veins opening into the pericardium, pericardial pouch, etc.; 15. Dissection of the abdomen of a female from the dorsal side to show the course of the superior abdominal artery; 16. Transverse section through the proximal region of the cephalic artery; 17. Transverse section through the pericardial pouch.

The *Pericardium* surrounding the heart is pentagonal and situated in between the inner corners of the fused thoracic epimera. Its posterior boundary is the anterior end of the rectangular projection from the anterior margin of the first abdominal segment. The postero-lateral boundaries are the inner edges of the eighth thoracic arthrophragm and they are 13 mm. long in a crab, 8 cm. across the carapace. The lateral boundary is the inner pointed corner of the fused thoracic epimera and is 8 mm. long. The anterior border is bounded by the middle portions of the gonads and the digestive glands and is 14 mm. The five branchio-cardiac veins on each side of the pericardium opens into it via three openings, the first two and the last two opening through single apertures. The last opening is situated at the corner between the lateral and postero-lateral borders of the pericardium. Although this opening is one, the partition between the two veins forming it, can be seen just behind the mouth of the opening.

*The Heart* (Text-figs. 11-13): It is a slightly greenish yellow, pentagonal body with all the sides of the pentagon concave. The two postero-lateral sides are more concave than the other sides and the anterior side which is 12 mm. in the above crab is the least concave of all the sides. An imaginary line connecting the tips of the lateral spines, will be just touching this anterior side of the heart. The two lateral sides are 6 mm. each and the two postero-lateral sides which are much concave, 9 mm. each in the same crab.

The heart is suspended inside the pericardium by means of bands of fibrous connective tissue, which are called *alae cordis* (Text-fig. 14). There are five paired and one unpaired *alae cordis*. The dorsal and ventral antero-lateral *alae cordis* are very close to each other, unlike that of *Cancer*, so that at the anterior side, the dorso-ventral border is very short and at side view it gives a triangular appearance as against rectangular in *Cancer* with only the ventral posterolateral *alae cordis* in the middle.

Three pairs of *ostia*, through which the blood enters the heart from the pericardium are present in the walls of the heart. One pair is situated in the dorsal wall, one in the centre of each half of the wall and is placed at an angle of 45° or 50° to the horizontal axis of the heart. The other two pairs are situated in the lateral walls of the heart, one pair in the middle of the triangular anterior lateral side and the other in the centre of the posterior ventrolateral side.

*The arteries*: There are two anterior paired and three median, one anterior, one descending downwards and the third posterior, arteries arising from the heart.

*Cephalic or ophthalmic artery*: (Text-fig. 14) is a median one arising from the middle of the anterior dorsal border of the heart. At its proximal region there is a swelling which extends to one-fourth of the whole length of the artery from its starting point from the heart to the portion where it bifurcates. The swollen portion is 4 or 5 times as broad as the artery itself. Transverse sections through this swollen portion (Text-fig. 16) show that it is a reticulate glandlike mass covering the wall of the artery, thus giving a swollen appearance to the portion of the artery. Pearson (1908) mentions a cellular mass in the neighbourhood of the cephalic artery above the foregut. These two may be the same. According to Cuenot (1895) it is a lymphatic gland in which the amoebocytes are formed.

The *lateral artery* (l. art.) arises from the anterior border of the heart slightly ventral to the region of the cephalic artery. The *hepatic artery* is outer to the lateral artery.

The *posterior aorta* or superior abdominal artery (Text-fig. 15) arises from the ventral middle portion of the heart, just behind the portion from which the sternal artery goes forwards. A pair of small vessels are given off one to each side, from the aorta just after it leaves the heart. Immediately anterior to the hindgut caecum another pair of branches, the *postero-lateral* arteries (pla) is given off. Each branch travels transversely towards the inner end of the eighth thoracic arthrophragm and near that it divides into two branches. The anterior branch immediately gives off one branch to the muscles of the coxopodite of the fourth peraeopod beneath the eighth thoracic arthrophragm. The main branch goes forwards and gives off branches to the muscles of the coxopodites of the other walking legs. The posterior branch immediately gives off one branch towards the inner side. This branch again divides into three, the central one going to the appendage of the first abdominal segment and the others going to the posterior ends of the digestive gland which ends at that region. The main vessel goes to the posterior region near the eighth thoracic arthrophragm and supplies the muscles extending from the abdomen to the cephalothorax etc. Of the two postero-lateral arteries, the one in the side where the hindgut caecum coil is situated, gives off a branch to the same,

Behind this the posterior aorta enters the abdomen. The arrangement of the aorta in the abdomen is not different in the two sexes, except for the reduction in the number of branches in the male. The aorta passes posteriorly above the hindgut and at the junction between the second and third abdominal somites it gives off a pair of branches to the appendage of that segment. Then the aorta gradually passes on to the right side of the abdomen and from the junction between the third and fourth somites it runs posteriorly on that side of the hindgut. It gives off three more pairs of branches to the third, fourth and fifth segments. Just after it gives off the last pair at the anterior region of the fifth segment it divides into two small branches of which one goes to the left side and runs posteriorly on that side and the other continues in the same side, giving off minute branches to the sides. In the male only two lateral branches are present.

*Sternal artery* (Text-Fig. 13. st.a): This artery leaves the heart at the ventral middle portion just in front of the starting point of the posterior aorta, and is the widest vessel starting from the heart. It passes downwards and forwards upto the foramen in the centre of the ventral nerve mass. Pearson calls the region upto the foramen as the descending artery. It passes down through the foramen and just below the foramen it passes forwards in a horizontal direction branching in the same way as in *Cancer*.

The blood sinuses and veins are identical with those of *Cancer*. The blood is also the same as that of *Cancer pagurus*. Coagulation of blood takes place on exposure to air. Tait (1911) has stated that at least three distinct modes of blood coagulation may be recognized in Crustacea, Coagulation A, B and C. According to him the coagulation of the blood of *Cancer pagurus* belongs to the type A and coagulation B is present in *Carcinus maenas, Palaemon serratus, Portunus puber, and Homarus vulgaris*. In *Neptunus sanguinolentus*, also coagulation B which consists in the agglutination of the blood corpuscles with subsequent general jellying of the blood plasma, as different from coagulation type A which is agglutination without subsequent jellying of the plasma, is noticed.

*The pericardial pouch* (Text-fig. 14): There is a pair of large, broad, flat and bag-like structures behind the postero-lateral corners of the pericardium. Its anterior, broader end is attached to the line where the horizontal eighth thoracic arthrophragm meets the fused epimera of the anterior thoracic somites and the inner end of this anterior border opens into the posterolateral corner of the

pericardium. It runs backwards and inwards above the eighth arthrophragm, becomes narrower and is inserted on the anterior edge of the first segment of the abdomen on both sides of the rectangular membranous portion of the first abdominal somite projecting into the posterior region of the thorax.

A transverse section of the pouch reveals that it is covered with a cuticle as mentioned by Pearson. The cuticle is 6 to 7μ in thickness. Below this cuticle is a layer of columnar epithelial cells, which are 48 to 52μ in length. Below the epithelium the whole cavity inside the pouch is filled with a reticulate cellular mass (Text-fig. 17) which is identical with that found in the proximal region of the cephalic artery. So this pericardial pouch also, probably, has the same function of a lymphatic gland, i.e., producing amoebocytes for the blood. The opening of the pouches into the pericardial sinus strengthens this view.

## EXPLANATION OF TEXT-FIGS.

acd 1-6—Alae cordis of the heart; aff—Afferent branchial sinus; agl—Antennary gland; al.l—Anterolateral lobe of the bladder; arp. 8—Eighth thoracic arthrophragm; brc 1-5—Branchiocardiac veins; bre—Branchial excretory cells; brg—Branchial glands; ca—Cephalic artery; ct—Connective tissue; cu—Cuticle; dost—Dorsal Ostium of the heart; ds—Dorsal spine of the gill lamella; eff—Efferent branchial sinus; ep—Epithelial cells; epd—Epidermis; epm 1-6—Epimera of the thoracic somites 1 to 6; e.tu—Epithelium of the renal tubule; f1—Flabellum of the 1st maxilliped; g1-8—Gills 1 to 8; h—Heart; ha—Hepatic artery; hgc—Hindgut caecum; hl—Hepatic lobe of the bladder; ibs—Interbranchial septum; itc—Intratubular tissue; itt—Intertubular tissue; l—Lamella; la—Labyrinth; l.art—Lateral artery; lls—Lamellar sinus; lost—Lateral ostium of the heart; ls—Lateral spine of the gill lamella; mdl—Median dorsal lobe of the bladder; mdstha—Musculus dorsalis superficialis thoraco-abdominalis; ml.l—Midlateral lobe of the bladder; mvl—Median ventral lobe of the bladder; ocm—Outer cellular membrane of the labyrinth; oe—Oesophagus; o.lls—Outer lamellar sinus; pc.ag—Concave region in the centre of the posterior face of the antennary gland; per—Pericardium; per.p—Pericardial pouch; pl—Posterior lobe of the bladder; pla—Postero-lateral artery; post—Posterior ostium; sa. a—Superior abdominal artery; st. a—Sternal artery; ur—Ureter.

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# Nutritional Requirements of Fungi*

BY

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I wish, at the outset, to thank the University of Madras for having done me the great honour of choosing me to deliver the Dr. Todla Ekambaram Endowment Lectures this time, and, to record my deep indebtedness to my teacher, Professor T. S. Sadasivan, for having made me worthy of this honour.

The aspect of physiology I have chosen for these lectures, *viz.*, nutrition, is nothing new, and is probably the most ancient and fundamental. Every organism, as is well known, must find in its environment both the structural units and the energy source for building and maintaining its living substance, protoplasm. These are termed its 'nutrients'. The nature of these nutrients or food substrates, however, varies with the different organisms and we are all familiar with the two classical early divisions comprising the 'autotrophic' and 'heterotrophic' organisms. This was based on their ability, or inability, to synthesize all of the vitally needed organic compounds from simple inorganic substances. In other words, it was the nature of the substrate used for the building up of protoplasm that weighed most in consideration. The autotrophs were considered synonymous with the photosynthetic green plants, and the heterotrophs with the nonphotosynthetic animals and microorganisms. With the tremendous advance in our knowledge, however, regarding the mechanism of nutrition in the various groups of organisms, we had to broaden our concept of nutritional classification of organisms, taking both the food substrate and the energy source into consideration. Based on the substrate, we have the LITHOTROPHS, utilizing inorganic compounds, and the ORGANOTROPHS, requiring at least certain performed organic

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substrates. Based on the energy source, we have the PHOTOTROPHS using radiant energy, and the CHEMOTROPHS, using chemical energy. Since for any organism both a food substrate and an energy source are necessary, we arrive at the recent classification, *viz.*, into PHOTOLITHOTROPHS (represented largely by the green plants), PHOTOORGANOTROPHS (the few photosynthetic bacteria), CHEMOLITHOTROPHS (certain highly specialized bacteria), and CHEMOORGANOTROPHS (animals and the great majority of microorganisms) (see Stanier *et al.*, 1958). The fungi, the organisms of particular interest here, fall under the last group.

The above classification does not mean that the basic nutritional necessities of the various groups of organisms differ. In fact, what all living things need, in the final analysis, are some 17 elements. Nevertheless, the form in which these are utilized differ among the different organisms and the nutritional classification referred to reflects precisely this difference in biosynthetic abilities of the various organisms.

The composition of protoplasm is too well known to warrant enumeration here. The need of organisms for those elements which go to form the structural components of protoplasm and hence consumed in comparatively large amounts is not difficult to visualize. For instance, analysis of the living matter from animals, plants or microorganisms, even when grown on different substrates, always reveals the presence of elements such as C, H, O, N, P, S, etc. These are the major components of the carbohydrates, proteins and fats, constituting the protoplasm, the cell walls and cell membranes. But, what has been more difficult to envisage and elucidate is the need for certain inorganic and organic substances in minute amounts, that rarely form any detectable part of the end products of metabolism. I would like to confine myself to a brief discussion of just two groups of these so-called 'micronutrients'—*i.e.*, those needed only in small amounts—in fungal nutrition, *viz.*, the vitamins and the trace elements.

Every living organism may be considered a gross catalyst, continually breaking down substrates and synthesizing cell material, with all the accompanying reactions necessary to keep these up. These processes, as we all know now, are carried out by the action of a host of vital catalysts, the enzymes, which are themselves proteins. These in turn seem to need still finer catalysts to 'activate' (used in a broad sense) them. It is here the micronutrients

would come into the picture, and this role accounts for the very minute quantities required of them.

### *THE VITAMINS*

Fungi, like all other organisms so far known, require minute amounts of specific organic compounds for growth. Organic substances required by a given organism in addition to its principal sources of carbon and energy are termed 'growth factors' for that organism. In its broadest sense, then, this term should include the amino acids, necessary for the synthesis of proteins, and the purines and pyrimidines, needed for the synthesis of nucleic acids, as well as the vitamins and some hormones. Nevertheless, it must be recognized that the amino acids and the purines and pyrimidines are incorporated into the structural constituents of protoplasm, whereas the vitamins seem to serve mainly as building blocks for the functional coenzymes which activate the vital catalysts. Understandably then, the latter will be needed only in much smaller amounts than the former.

The characteristic features of a growth factor have been defined by Lilly and Barnett (1951) as: (1) its organic nature, (2) its activity in minute amounts, (3) its catalytic action and (4) the specificity of its action. The vitamins satisfy all these.

Among the fungi, the yeasts have been the most studied with regard to vitamin requirements. It was Schopfer's work in 1934 on *Phycomyces blakesleeanus* and thiamine that gave the necessary impetus for further intensive studies on the vitamin needs of the filamentous fungi. Much work has been done since then on the vitamin requirements of naturally occurring fungi, and more recently those of induced mutants, both types of material having provided valuable information regarding the biosynthesis as well as function of vitamins in living things generally.

Many fungi are able to grow and develop normally on a substrate containing no added vitamins, and the addition of a few vitamins may not appreciably affect their growth (the generally considered criterion of nutrition), as for instance, species of *Aspergillus*, *Penicillium* and *Fusarium*. One may surmise that these either do not need the vitamins, or, can synthesize them to meet their need. The growth of other organisms which need an external supply of vitamin (s) on culture filtrates of these fungi (growth of *Piricularia oryzae* on culture filtrate of *Fusarium vasinfectum* (Apparao, Subba Rao and Suryanarayanan, 1955)) is proof that they

synthesize the vitamins, even in amounts greater than their own requirements. Such fungi are called SELF-SUFFICIENT OR AUTOTROPHIC WITH RESPECT TO VITAMINS. On the other hand, those fungi which are unable to synthesize the vitamins they need in metabolism are termed VITAMIN-DEFICIENT, or HETEROTROPHIC WITH RESPECT TO VITAMINS (Schopfer, 1943).

Vitamin deficiency studies are not difficult to perform, but they must be carried out under carefully controlled conditions, using clean glassware, pure chemicals and water, and precaution against vitamin contamination from other sources, such as lint from the cotton plugs etc. Despite all precautions possible, variable results often occur, and tests may need to be repeated several times before vitamin deficiencies of some fungi can be definitely determined.

Vitamin deficiencies among fungi have been detected mainly for certain members of the water-soluble B-complex group. The most common vitamins involved are thiamine, biotin, inositol, pyridoxine, nicotinic acid, and pantothenic acid. Although for preliminary screening experiments test-tube slant cultures using pyridine-purified agar can be conveniently used, for more critical studies a pure synthetic liquid medium is ideal and desirable in that it also facilitates more accurate measurement of growth or other physiological activities of the fungus. The preferred method of discovering a need for any substance is to grow the organism concerned in a medium adequate in all respects but essentially free from the substance under study; *i.e.*, in the case of vitamins (as with other essential nutrients), by omitting one vitamin from a mixture of the known biologically important ones. Nevertheless, since there are reports that some vitamins for which there may not be a 'requirement' (*i.e.*, need for an external supply), if added to the medium, may have limiting effects on growth and development, it is quite a worthwhile practice to add the vitamins singly and in combinations of two, three or more to the medium and note the response of the organism.

Although a number of fungi are known to be vitamin-deficient, the needs of the various species differ. This is of course as it should be since variation, rather than uniformity, is the rule among organisms. There are different types of vitamin deficiencies in fungi. Fungi such as *Phycomyces blakesleeanus, Sclerotium oryzae* and species of *Phytophthora* and *Pythium,* show a requirement for thiamine only; the classical *Neurospora* wild type requires only biotin. These are known as SINGLE VITAMIN DEFICIENCIES. On the other

hand, *Piricularia oryzae* (thiamine & biotin), species of *Ceratostomella* (some needing thiamine & pyridoxine, others needing biotin also), *Diplocarpon rosae* (thiamine & inositol), *Sclerotinia camelliae* (thiamine, biotin & inositol), *Penicillium digitatum* (thiamine, biotin, pyridoxine & pantothenic acid) and a strain of *Blastocladia pringsheimii* (thiamine, biotin, nicotinic acid & *p*-aminobenzoic acid) have need for two or more vitamins, in varying combinations and concentrations. Such deficiencies are called MULTIPLE VITAMIN DEFICIENCIES. These deficiencies may be TOTAL, *i.e.*, no growth occurs without added vitamins, as in the case of *Phycomyces blakesleeanus* and thiamine, or, PARTIAL, allowing a subnormal or delayed growth in the absence of vitamins, as for instance, *Lenzites trabea* and thiamine. Again, the deficiency may be ABSOLUTE, no growth occurring under whatever conditions tried, e.g., *Phycomyces blakesleeanus* and thiamine, or CONDITIONED, by factors such as pH, or salt concentration of the medium, or temperature of incubation. For example, *Sordaria fimicola* can synthesize its own thiamine if grown at initial pH 4·4, but requires an external supply at pH 3.8; a mutant of *Neurospora,* requires riboflavin only above 25° C.; *Pythium butleri* requires thiamine only in high salt medium; the requirement for inositol of *Sclerotinia camelliae* seems to be temperature-controlled. Other types of conditioned deficiency include: (1) The reduction or elimination of a vitamin requirement by supplying a metabolite which is necessary for the synthesis of the vitamin (such as replacement of nicotinic acid by large amounts of tryptophan which is considered a precursor), or, a metabolite for the synthesis of which the vitamin is necessary (replacement of *p*-aminobenzoic acid by methionine; of biotin by aspartic acid). (2) The need for a vitamin is more acute at a particular stage of development. This latter type is the least explored and investigations on this aspect are bound to be rewarding. It is quite possible that germinating spores may require factors which the mature mycelium does not. A single fungus may show a combination of these various types of vitamin deficiency. Examples for all these can be multiplied to a large extent numerically, but the few that have been cited here would have given us an idea of the nature of vitamin deficiencies in fungi (see Lilly & Barnett, 1951; Cochrane, 1958).

By far the majority of vitamin-deficient filamentous fungi has been shown to require thiamine (as is obvious even from the limited number of examples given above), immediately followed by biotin-requiring fungi. The need for pyridoxine and inositol is

less frequently noticed, and there are but rare reports of a requirement for nicotinic acid, *p*-aminobenzoic acid and pantothenic acid. The recent report that *Venturia inaequalis* requires folic acid and ascorbic acid (Fothergill & Ashcroft, 1955), the latter to a greater extent than the former is indeed interesting particularly in view of the reported absence of ascorbic acid oxidase among fungi and doubt about any role of ascorbic acid in their metabolism (see Cochrane, 1958). Among the yeasts, the most studied for vitamin deficiencies, biotin deficiency seems to be the most common, followed by thiamine, pantothenic acid, inositol, nicotinic acid and pyridoxine. There appears to be only a single report of *p*-aminobenzoic acid requirement among the yeasts (see Lilly & Barnett, 1951).

It is generally assumed that a fungus (or other organism) which is independent of an external supply of vitamin is so by virtue of its ability to synthesize the same. This assumption is borne out by the entire experimental evidence so far available. The utilization of thiamine and its moieties (pyrimidine and thiazole) as growth factors by some organisms provides an excellent illustration of the correlation between the biosynthetic abilities and nutritional requirements of different organisms. Some fungi (eg., *Sclerotium oryzae*) lack the capacity to synthesize the pyrimidine moiety, but if supplied with this it can combine it with the self-synthesized thiazole and form the thiamine molecule; others (*Mucor ramannianus*) can synthesize pyrimidine, but need thiazole supply to form the whole unit; yet others (*Piricularia oryzae* or *Phycomyces* spp.) have only the combining capacity and need to be supplied with both the moieties; some (*Phytophthora* spp.) have none of these abilities and hence require the intact thiamine. Those fungi which are self-sufficient for thiamine can synthesize as well as combine the two moieties to form thiamine, and hence need no external supply. Even the deficiency for the moieties may be conditioned, for instance, *Piricularia oryzae*, which normally needs a supply of both the moieties, shows a need for pyrimidine alone when maltose forms the carbon source (Suryanarayanan, 1958). In all these cases, however, the final functional unit is the intact thiamine molecule, and all can utilize the complete vitamin when supplied.

In recent years, the emphasis in vitamin studies has shifted considerably, from just demonstrations of deficiencies under one or another condition, to an understanding of the functions and

modes of action of these growth factors. A number of vitamins, such as thiamine and nicotinic acid, have been shown to perform definite functions in fungi as well as animals, and there is no reason to assume that their fundamental functions in these two groups of chemoorganotrophic organisms are essentially different. In fact, the general assumption that fungi not requiring an external supply of vitamins are self-sufficient for them has stemmed from the premise, at least as regards the water-soluble B-vitamins, that the known vitamins have essential functions in all cells. Further evidence for the essentiality of vitamins in fungus physiology is provided by the production of vitamin-deficient mutants from wild types which do not show such deficiency; eg., the classical biochemical mutants of *Neurospora crassa,* and species of *Aspergillus* and *Penicillium.*

Vitamins synthesized by a fungus usually appear in the medium and are detected there by bioassay. Nevertheless, in *Penicillium chrysogenum* (thiamine, riboflavin & nicotinic acid), *Aspergillus niger* (riboflavin) and some other fungi (thiamine), they remain in the cell. In *Erymothecium ashbyii* and *Ashbya gossypii* the esters of riboflavin (especially FAD) occur in the cell while free riboflavin is found in the medium.

In the following discussions I would like to stress the functional aspect of vitamin nutrition. After 1930, a striking parallelism began to emerge between the vitamins and coenzymes, and as new vitamins were isolated they were often found to be parts of coenzymes. This association is so general that today we routinely assay a new vitamin for coenzyme activity and a coenzyme for vitamin activity. In all cases where the functional forms of the vitamins are known, they have been shown to be coenzymes for specific enzyme systems.

A word of clarification in terminology may not be out of place here. It is often difficult to distinguish between what is meant by the terms 'coenzymes', 'prosthetic groups' and 'activators' of enzymes. Perhaps it is best to reserve the term 'coenzyme' for the organic fragments that are not normally isolated with the apoenzyme, i.e., the dissociable fragments. A prosthetic group would then represent a firmly attached coenzyme, such as iron protoporphyrin or the flavin part of the flavoproteins, and 'activator' (in this restricted sense) might be reserved for metals etc. At any rate, all these substances are generally of low molecular weight, dialyzable and heat stable.

Neilands and Stumpf (1958) have recently classified the known coenzymes into two groups, *viz.*, hydrogen-carrying and group-carrying coenzymes. To this they have added a third group, of 'vitamins which have not yet been promoted to the status of coenzymes', called 'cofactor group'. (The intimate association between vitamins and coenzymes is obvious in this classification.) This is a convenient classification to understand vitamin function.

### VITAMINS INVOLVED IN HYDROGEN TRANSPORT

*Nicotinic acid* (*niacin, antiberiberi factor*): The amide form of this vitamin, nicotinamide, forms the base for the two important coenzymes, di- and triphosphopyridine nucleotides, abbreviated as DPN and TPN. The free acid, the amide and the co-enzymes are all widely distributed in nature. The main function of these coenzymes is to transport hydrogen from metabolites to the carrier of next highest oxidation-reduction potential, usually the flavoproteins. They are involved at least indirectly in oxidative phosphorylation. Some of the enzymes having the pyridine nucleotides as coenzymes are: alcohol dehydrogenase (DPN), aldehyde dehydrogenase (TPN), glucose-6-phosphate dehydrogenase (TPN), glyceraldehyde-3-phosphate dehydrogenase (DPN) and isocitric dehydrogenase (DPN).

The mechanism of action of these coenzymes may be pictured as a reversible reduction of the pyridine ring. Under physiological conditions, the reduced pyridine nucleotide is reoxidized by the flavoprotein systems.

Glucose-6-phosphate + $TPN^+ \rightarrow$ Gluconic acid-6-phosphate + TPNH + $H^+$.

TPNH + $H^+$ + Flavoprotein (ox) $\rightarrow$ $TPN^+$ + Flavoprotein (red).

The following series of reactions are involved in the formation of the coenzymes from nicotinamide:

Nicotinamide + Ribose-1-phosphate $\rightarrow$ Nicotinamide riboside + $H_3PO_4$. Nicotinamide riboside + ATP $\rightarrow$ Nicotinamide mononucleotide (NMN).

NMN *is further* phosphorylated by ATP *to form the two* functional pyridine nucleotides:

NMN + ATP $\rightarrow$ DPN + Pyrophosphate.

DPN + ATP $\rightarrow$ TPN + ADP.

The enzymes catalyzing these reactions have been isolated from yeasts.

*Riboflavin (lactoflavin, vitamin $B_2$)*: This vitamin forms the base for the other important group of hydrogen-carrying coenzymes, the flavin nucleotides, FMN and FAD. These are the prosthetic groups for many yellow flavoprotein enzymes, such as the old and new yellow enzymes, nitrate reductase, xanthine oxidase and cytochrome c reductase, involved in biological oxidation-reduction reactions. In the oxidation of glucose-6-phosphate by TPN, FMN accepts the hydrogen from the reduced pyridine nucleotide and gives it away to other electron acceptors such as oxygen.

Riboflavin is produced commercially from *Erymothecium ashbyii*, a fungus which produces so much of the vitamin that it crystallizes out in the medium. This was the first vitamin for which a suitable bacterial assay could be worked out (Snell & Strong, 1939). FMN, the first coenzyme to be chemically synthesized, is obtained from riboflavin by direct phosphorylation. The biological conversion of riboflavin to flavin phosphates, the biologically active forms, appears to be effected by an enzyme-catalyzed reaction in which ATP (adenosine triphosphate) serves as the phosphorylating agent:

Riboflavin + ATP→Riboflavin-5-phosphate (Flavin mononucleotide — FMN) + ADP. (It is because riboflavin-5-phosphate is composed of a base (dimethylisoalloxazine), a sugar derivative (ribitol) and phosphate that it is termed a mononucleotide, but this is somewhat erroneous since the sugar residue is the corresponding alcohol of ribose.) One molecule of FMN is linked to a molecule of AMP (adenosine-5-phosphate) to form flavin adenine dinucleotide or FAD. It is probably synthesized in biological systems by the reaction of FMN with ATP, catalyzed by the enzyme FAD synthetase.

The mode of action of these coenzymes in oxidation-reduction reaction is conjectured to be by the reversible reduction of the isoalloxazine nucleus.

## Vitamins Involved in Group Transport

*Pantothenic acid*: This was the first vitamin to be discovered, isolated and characterized solely by work with microorganisms, an achievement which can be attributed almost

exclusively to one man, R. J. Williams. This vitamin forms a constituent of the important acyl group-carrying coenzyme, Coenzyme A or CoA. The occurrence of CoA in nature parallels that of pantothenic acid. Interest in the coenzyme increased when it became apparent that it functions generally as a carrier of the acetyl group. The first concentrates of CoA contained no detectable amounts of any of the known vitamins including pantothenic acid. On acid hydrolysis, however, large amounts of β-alanine appeared. Finally, Lipmann and his group were able to liberate pantothenic acid from the coenzyme by hydrolysis with two enzymes, a liver enzyme and alkaline phosphatase. The fact that CoA had to be hydrolyzed with a phosphatase in order to liberate the vitamin activity for the test bacteria proved the coenzyme to contain phosphate.

The ultimate step in pantothenate synthesis is known to be the coupling of pantoic acid and β-alanine. (There is some evidence that α-ketoisovaleric acid is a precursor of pantoic acid.)

Pantoic acid + β-alanine + ATP→Pantothenic acid + AMP + pyrophosphate. From pantothenic acid and cysteine in the presence of ATP, pantetheine is formed. Pantetheine is phosphorylated by ATP in the presence of 'pantetheine kinase' to yield 4-phosphopantetheine, which reacts with ATP in a reaction catalyzed by 'dephosphoCoA pyrophosphorylase' to give dephosphocoenzyme A. This is further phosphorylated with ATP to give rise to coenzyme A or CoA, the final functional form of the vitamin.

The mechanism of action of CoA centers on its terminal sulfhydryl group, which can be alternately acetylated and deacetylated, just as the pyridine ring of the pyridine nucleotides can be alternately oxidized and reduced. The acetyl-CoA compounds thus formed are important and essential intermediates in cellular respiration, in fat utilization and fat synthesis, and in the synthesis of sterol compounds.

The importance of the TCA (tricarboxylic acid, or, the citric acid) cycle in the metabolism of aerobic organisms needs no special emphasis, though in many microorganisms alternative pathways of carbohydrates exist. Into the TCA cycle is channelled a major portion of the terminal oxidation of tissue components. By means of this cycle the products of fat, sugar and amino acid metabolism are eventually burned to $CO_2$ and water. The chemical energy released in the oxidation of these substances is captured in part as chemical bonds of high energy content.

The physiological efficiency of the cycle demands that the cycle works in the direction of citrate-α-ketoglutarate-succinate-oxalacetate, which is exergonic, and not the reverse which would be endergonic and hence uneconomical. Indeed, a significant aspect of the citric acid cycle is that the equilibrium in the oxidative decarboxylation of α-ketoglutaric acid is so far in the direction of succinic acid as to make the operation of the cycle essentially unidirectional. An examination of the sequences in this cycle reveals that CoA participates in it at two vital points, *viz.*, in the condensation of oxalacetate to citrate, involving acetyl-CoA, and the decarboxylation of α-ketoglutarate to the important respiratory substrate, succinate, involving succinyl-CoA. One cannot resist the temptation to state that it is CoA, acting as the acyl-donor, that starts the citric acid cycle as well as keeps it going in the physiologically efficient direciton.

In view of the importance of CoA in the metabolism of carbohydrates, fats and nitrogen compounds, the activity of pantothenic acid as a vitamin is obvious. More than three fourths of all the carbon in an organism seems to be incorporated in this coenzyme (Coenzyme A) at some stage or other (see Butler & Downs, 1960).

*Pyridoxine (Vitamin $B_6$ or adermin)*: This vitamin is the parent substance for pyridoxal phosphate, the functional coenzyme for enzymes of amino acid metabolism. The aldehyde and amine analogues, pyridoxal and pyridoxamine, probably occur in nature in considerably greater concentration than pyridoxine, mainly as the phosphates. Pyridoxal phosphate takes part in the decarboxylation of amino acids, transamination between amino and keto acids, racemization of amino acids, deamination of hydroxy amino acids and desulfhydration of sulfur-containing amino acids, as well as miscellaneous transformations of the amino acids. The accumulation of nitrite from nitrate in pyridoxine-less cultures of *Neurospora crassa* mutants indicates a role of the coenzyme in nitrite reduction.

The mechanism of action of these coenzymes is not definitely established, and a pure transaminase has not yet been isolated. There is, however, good evidence that an intermediate Schiff base may be formed by the aldehyde form and an amino acid, the combination being effected between the aldehyde group of the coenzyme and the amino group of the amino acid with release of one molecule of water. This intermediate would then decompose into the corresponding keto acid and the amine form of the coenzyme.

This amine group may then be transferred to another keto acid with the reformation of the aldehyde form of the coenzyme.

*Thiamine (Vitamin $B_1$ or aneurin)*: This was the first vitamin to have been studied as a known entity in fungal nutrition. The metabolically active form is the pyrophosphate, thiamine pyrophosphate (TPP), or diphospho-thiamine, long known as cocarboxylase, involved in the decarboxylation of pyruvic acid to acetaldehyde and $CO_2$. In fungi it has been shown to accelerate ethanol formation through pyruvic acid decarboxylation, and when absent leads to accumulation of the keto acid.

TPP participates not only in carboxylase activity, but also in the enzymic decarboxylation of $\alpha$-ketoglutarate (to succinate) and other keto acids, and in the reaction catalyzed by transketolases. The enzyme transketolase has been crystallized from yeast and participates in the conversion of pentose phosphate to hexose phosphate. (The pentose phosphate pathway is an important alternate pathway in carbohydrate metabolism in microorganisms.) In the reaction, a ketol group is transferred from xylulose-5-phosphate (pentose phosphate) to a suitable acceptor aldehyde. The crystalline enzyme contains TPP as an essential constituent.

Although all the enzymic reactions that require the participation of TPP appear to involve the initial conversion of the substrate to an 'active aldehyde' that is either released as a free aldehyde (acetaldehyde) or is transferred to a suitable acceptor (as in formation of acetoin), the chemical mechanism whereby TPP facilitates these transformations is obscure.

Until recently, it has been thought that the mechanism of action of TPP was by the opening and closing of the thiazole ring, inasmuch as a sulfhydryl group appears during the process. From more recent work (Breslow, 1957; Downes & Sykes, 1957—cited by Neilands & Stumpf, 1958), however, it would appear that the electron-rich C-2 of the thiazole portion may form a complex with an $\alpha$-keto acid which immediately rearranges to yield $CO_2$ and a carbonyl-thiazole product. This latter may then dissociate to give the aldehyde and thiamine (see Neilands & Stumpf, 1958).

Although it might be assumed that the biosynthesis of thiamine proceeds via coupling of the two moieties, pyrimidine and thiazole, in *Neurospora crassa* it does not seem to be so simple, and the following reactions are proposed:

Pyrimidine+unidentified thiazole precursor→thiamine-like intermediate→thiamine. Direct coupling of the moieties may occur as a minor pathway (see Cochrane, 1958). In this connection, it is interesting to recall the observation of Robbins and Kavanagh (1941) that *Phycomyces blakesleeanus* destroyed thiazole more rapidly than it did the pyrimidine moiety, and that an excess of thiazole in the mixture was more effective than equal quantities, which they called the 'thiazole effect'.

### VITAMINS FOR WHICH FUNCTIONAL FORMS ARE NOT YET KNOWN

*Biotin (Vitamin H)*: Although one of the earliest of the 'bios' factors to be studied, biotin still falls under this category. Soon after its isolation, it has been demonstrated to be identical with 'coenzyme R'—considered to have a stimulatory effect on respiration of non-growing *Rhizobium;* nevertheless, neither the nature of this so-called coenzyme nor its exact biochemical action has yet been established (see Fruton & Simmonds, 1958).

Biotin is believed to be involved directly or indirectly in at least the following phases of microbial metabolism:

1. Somewhere during synthesis of aspartic acid from pyruvic acid, via oxalacetic acid, and possibly in the metabolism of other nitrogen-containing compounds;
2. In deamination reactions, for instance, of aspartic acid to oxalacetic acid, and of serine to pyruvic acid;
3. In decarboxylation reactions; e.g., of oxalacetic acid to pyruvic acid and $CO_2$, and of oxalosuccinic acid to $\alpha$-ketoglutaric acid and $CO_2$;
4. In the synthesis of oleic acid.

Since biotin is required in very minute amounts (the absolute requirement is usually less than 5 $\mu$g. per liter), it may turn out that it is indirectly involved in the synthesis itself of enzymes. There is some evidence that it may be involved in the synthesis of long-chain fatty acids.

It is quite possible that when the actual functional form of biotin becomes known, it may belong to the hydrogen-carrying group of coenzymes, since it has been suggested that the various deamination and decarboxylation reactions cited above involve an inter- or intramolecular hydrogen transfer in which the functional biotin molecule gets reversibly reduced (Sebrell & Harris, 1954).

The biosynthesis of biotin may be summarized as:

Pimelic acid → desthiobiotin → biotin.

Some fungi, such as *Neurospora crassa* and *Ceratostomella ips*, can utilize desthiobiotin, whereas some others, such as *C. pini* and *Sordaria fimicola,* need biotin itself.

*Inositol*: Although certain fungi are known to be deficient for this compound, it is doubtful whether this should be considered a vitamin in the usual sense. Its structure itself differs from that of other vitamins and resembles more that of sugars. Moreover, no cofactor essential for enzymic reactions has been shown to contain this compound as a structural unit, nor is any coenzyme function known. Most of the inositol of *Neurospora crassa* mycelium has been found associated with phospholipids, and this suggests that it is rather a structural component of the cell than a functional unit. Furthermore, the magnitude of inositol requirement is much greater than that of the other vitamins, again suggesting a structural rather than a catalytic role. It seems logical therefore to exclude inositol from the list of B vitamins and consider it along with some of the other growth factors such as amino acids or the purines and pyrimidines.

P-*Aminobenzoic acid* (PABA): Most of the interest in this compound used to revolve round its antagonistic action to sulfanilamides. Woods in 1940 observed that the bacteriostatic effect of sulfanilamide was reversed by *p*-aminobenzoic acid, a compound not previously known to have any biochemical function. It was this observation that gave the concept of 'competitive analogue-metabolite inhibition'. Sulfanilamide, being a structural analogue, is thought to interfere in enzyme reactions involving PABA. This hypothesis received support from subsequent work on the biological action of PABA.

Although the folic acid molecule contains the structure of PABA, it seems doubtful that the main function of PABA is in the formation of folic acid. For example, folic acid does not replace PABA for *Neurospora crassa*. PABA is considered to function in the transport of 1-C compounds, particularly the methyl groups, between amino acids; for instance, in the reversible demethylation of methionine. The fact that methionine replaces PABA for a mutant of *Neurospora crassa* supports this suggested function.

*Folic acid* (PGA *or Pteroylglutamic acid*): This vitamin is also known variously as vitamin $B_c$, *Lactobacillus casei* factor (LCF) and folacin. The only fungus shown to be stimulated by this vitamin is *Venturia inaequalis* (as previously mentioned). As noted above, the vitamin contains PABA structure and indeed many authors prefer to group PABA, folic acid and a variable number of glutamic acid residues into one family, the folic acid family (see Neilands & Stumpf, 1958; Fruton & Simmonds, 1958).

Folic acid has been shown to be involved in the transport of 1-C compounds in the synthesis of purines and Pyrimidines as well as amino acids (see Fruton & Simmonds, 1958). Although the role of PGA derivatives as carriers of 1-C units has been studied extensively, the nature of the functional form of the vitamin is uncertain. The available evidence indicates, however, that tetrahydro-PGA, rather than PGA itself, is the precursor of the active cofactor.

*The vitamins $B_{12}$*: These comprise a family of compounds, the chemical and biological relationships of which are not fully understood. Vitamin $B_{12}$, cyanocobalamine, was first discovered and its structure is now known. It is the only vitamin so far known to contain a metal, cobalt. Other cobalamins can be converted to it by cyanide treatment.

Vitamin $B_{12}$ is believed to function in the cell in the synthesis of 1-C fragments, particularly the labile methyl group.

## The Newer Vitamins

*The vitamins K* (*Koagulations*): Vitamins $K_1$ and $K_2$ are the only naturally occurring forms of vitamins K that have been completely characterized, and these are related to the synthetic form $K_3$ or menadione. Some compounds structurally related to vitamin K are fungitoxic. Menadione competitively inhibits their toxicity. This, however, is considered as insufficient evidence for a role of K vitamins in fungal metabolism (see Cochrane, 1958).

*Thioctic acid* (*Lipoic acid*): Studies on the nutrition of lactic acid bacteria begun many years ago by W. H. Peterson at the Wisconsin University led to the discovery of several new and interesting compounds with vitamin activity. Thioctic acid is the most recent addition to the list. The new vitamin is chemically a rather simple compound, the carbon skeleton of which is related

to *n*-octanoic acid, and is made available by routine organic-chemical synthetic methods.

The mechanism of action of lipoic acid would seem to be in some way concerned with the opening and closing of the cyclic disulfide ring.

The aerobic conversion (oxidative decarboxylation) of pyruvate to acetyl-CoA in which DPNH is oxidized via the cytochrome system represents a key step in the metabolic oxidation of glucose to $CO_2$ and $H_2O$. Lipoic acid is now known to be an essential co-factor in this important reaction. Although the enzymes participating in the conversion of pyruvate to acetyl-CoA have not yet been purified extensively, the available data are consistent with the following sequence of reactions.

During decarboxylation of pyruvic acid, an activated acetaldehyde-TPP compound is formed; by reaction of lipoic acid with this compound, acetyldihydrolipoic acid is formed, involving the reductive cleavage of the disulfide bond with the concomitant formation of an S-acetyl group. Enzymic transfer of the S-acetyl group to CoA gives acetyl-CoA and reduced lipoic acid. The reduced lipoic acid is oxidized by DPN to the disulfide form. The reduced pyridine nucleotide can be oxidized either by oxygen or another molecule of pyruvate itself, and thus the conversion of pyruvate to $CO_2$ and acetyl-CoA can be effected in the presence of catalytic amounts of TPP, lipoic acid and DPN. It is noteworthy that in addition to pantothenic acid, three other vitamins—thiamine, lipoic acid and nicotinamide—are related to participants in enzymic conversion of pyruvate in biological systems.

*Ascorbic acid (Vitamin C)*: Cochrane (1958) expressed doubt whether ascorbic acid has any role to play in fungal metabolism. The demonstration of a definite need for this vitamin for *Venturia inaequalis* (Fothergill & Ashcroft, 1955), however, does call for greater attention to this point, although the enzyme ascorbic acid oxidase has not been isolated from fungi. It is quite possible that by virtue of its strong reducing properties, it may serve as an intracellular reducing agent; for instance, in reactions involving $Fe^{2+}$ participation.

Tables I and II summarize our present knowledge regarding the functions and functional forms of the substances of established vitamin activity. It will be noted that wherever the functional

TABLE I

*Vitamins for which functional coenzymes are known*

| *Vitamin* | *Coenzyme* | *Function* | *Composition* |
|---|---|---|---|
| Nicotinic acid (amide form) | Pyridine nucleotides | Hydrogen transport | |
| | DPN | „ „ | Adenylic acid; NMN. |
| | | | „ ; $2PO_4$ |
| | TPN | „ „ | „ ; $3PO_4$ |
| Riboflavin | Flavin nucleotides | Hydrogen transport | Isoalloxazine; ribitol. |
| | FMN | „ „ | „ „ |
| | FAD | „ „ | Isoalloxazine; adenosine monophosphate |
| Pantothenic acid | Coenzyme A | Acyl group transport | Adenosine-3, 5-di $PO_4$; phosphopantetheine |
| Pyridoxine | Pyridoxal phosphate | Transamination; decarboxylation, etc. | Pyridoxal; $PO_4$ |
| Thiamine | Thiamine pyrophosphate (cocarboxylase) | Decarboxylation of ketoacids, &c. | Pyrimidine; thiazole; pyrophosphate. |

TABLE II

*Vitamins for which functional forms are not yet known*

| *Vitamin* | *Coenzyme* | *Function* |
|---|---|---|
| Biotin | ? | Deamination, decarboxylation. |
| Lipoic acid | ? | Hydrogen transport |
| Inositol | ? | Considered structural. |
| *p*-Aminobenzoic acid | ? | Transport of 1-C fragments (methyl gr.) between animo acids |
| Folic acid | ? | Transport of 1-C fragments to form purines and pyrimidines as well as amino acids. |
| Vitamin $B_{12}$ | ? | Synthesis of 1-C compounds. |

form of the vitamin is known it has been shown to be a phosphorylated compound, and we have every reason to believe, in view of the key position phosphorus transformations occupy in general metabolism, that the functional forms of the remaining vitamins also, when elucidated, may turn out to be phosphorylated compounds.

Considering their functions, biotin (since the reactions catalyzed by it has been explained as inter- or intramolecular hydrogen transfer) and lipoic acid can be placed under the hydrogen-carrying group, and PABA and folic acid under group-carrying category of Neilands and Stumpf's classification of vitamins. Inositol is better removed from among the vitamins for reasons already discussed. In view of the fundamental nature of the various biological reactions in which the vitamins are involved, the importance of these in the nutrition of fungi, be they autotrophic or heterotrophic with respect to vitamins, is indeed obvious.

THE TRACE ELEMENTS

The other group of micronutrients I would like to deal with include those metallic elements which are needed in minute amounts by fungi. Since we are dealing with the basic elements themselves here, there is no question—at least as far as we know now—of difference among fungi in synthesizing them. Nevertheless, fungi do differ in their ability to utilize the compounds in which these elements occur, as well as in their absolute requirements for

them. Some fungi such as *Aspergillus niger* may absorb them from normally insoluble compounds, and even utilize metallic zinc, whereas the majority depends on some soluble form or other.

The status of trace elements in fungal metabolism underwent a most spectacular change when Robert Armstrong Steinberg (1919a), in his epoch-making work, disproved Pffeffer's 'theory of chemical stimulation' and established instead the nature of some of them as indispensable nutrients for *Aspergillus niger*. Although their essentiality in plant nutrition has been claimed much earlier (Raulin, 1869; Bertrand, 1905), the idea was hardly accepted by plant physiologists of that time. Why this was so, and how effectively the earlier concepts, such as the 'Arndt-Schulz law', 'the oligodynamic effect' and 'the theory of chemical stimulation,' held sway in those times, are admirably described by Foster (1949). Since Steinberg's classical research, much useful work has been done, and the trace elements are now looked upon, not as spurious 'stimulants', but as biologically essential nutrients.

The controversy regarding the essentiality of trace elements in fungal nutrition has been due to ignorance of the probable sources of contamination. All the experimental materials normally employed, *viz.*, chemicals, distilled water, glassware and inoculum, contribute to the nature and amount of these contaminations. Although the pioneers in this field have had a hard task to convince their contemporaries and their immediate followers, at the present day, the need for extreme care and refined techniques in trace element essentiality studies is appreciated by all critical workers. The situation is quite comparable to vitamin deficiency studies, where the effect of these growth factors is often masked by contaminations from the ordinary culture materials used. The difficulties seem all the more accentuated in trace element work, since consistent trace element deficiencies show up only in RIGOROUSLY PURIFIED SYNTHETIC MEDIA, whereas vitamin deficiencies can be demonstrated in media using 'chemically pure' substances. For trace element studies, these compounds are far from being 'biologically pure'. The possible reason seems to be simple in that the trace elements tend to occur as impurities in combination with other substances, even after recrystallization, much more frequently than vitamins, which are themselves complex organic compounds. The special problems and techniques involved in trace element essentiality studies with fungi have been dealt with in detail earlier (Saraswathi-Devi, 1958c).

*Aspergillus niger* has been the most studied species among fungi for mineral nutrition. This organism has been shown to require Fe, Zn, Cu, Mn and Mo, by many workers. In the absence of any one of these from an otherwise adequate medium, typical deficiency symptoms are developed. Indeed, the need for these elements is so well established that some stable strains are now widely used as standard biological test organisms (see Saraswathi-Devi, 1954; 1958b). Other organisms that have been studied in this regard include *Rhizopus nigricans, Aspergillus flavus, Ceratostomella ulmi, Trichophyton interdigitale, Phymatotrichum omnivorum* and some fungal pathogens of tobacco. Using the elegant technique of bioassay for the suitability of the experimental conditions, *viz.*, by growing the 'M' strain (or Mulder's strain) of *Aspergillus niger*, the essentiality of Fe and Zn for nine species of *Fusarium* was proved (Saraswathi-Devi, 1958d). These elements could not be replaced in entirety by two of the most commonly needed vitamins, thiamine and biotin, either for the 'autotrophic' *Fusarium vasinfectum* (Saraswathi-Devi, 1956), or for the 'heterotrophic' *Piricularia oryzae* (Apparao, Saraswathi-Devi & Suryanarayanan, 1955). It was observed that these species did not need more than the left-over traces of Cu and Mn in the purified medium for normal growth.

Calcium, a macronutrient element in the nutrition of higher plants, is only a micronutrient for fungi. Species reported to need this element are very few, such as *Rhizoctonia solani, Sclerotium rolfsii* (Steinberg, 1948) and *Chaetomium* spp. (Basu, 1951).

Apart from the above-mentioned elements, gallium and scandium have been claimed to be essential for *Aspergillus niger* under some special experimental conditions (Steinberg, 1938, 1939). Since vanadium was present in the ash of many fungi analyzed, Bertrand (1943) held it to be essential for fungi in general. Nevertheless, these observations remain to be confirmed yet.

From the essentiality point of view, growth and sporulation have been the first considered criteria. There exists, however, a volume of literature to show that many other individual metabolic processes, such as production of acids, enzymes, pigments, vitamins and antibiotics, are profoundly influenced by trace elements (see Perlman, 1949; Foster, 1949). Nevertheless, the absolute indispensability of these elements for their production has been shown only in very few instances. The necessity of Fe for citric acid produc-

tion by *Aspergillus niger* (Perlman, 1947; Shu & Johnson, 1947), of Fe, Zn, and Mn for optimum production of penicillin by *Penicillium chrysogenum* (Jarvis, 1946; Jarvins & Johnson, 1947; cited by Perlman, 1949), of Zn for the production of antibiotics by *Fusarium hyperoxysporum* (Texera, 1948) and *Fusarium vasinfectum* (Kalyanasundaram & Saraswathi-Devi, 1955), of Cu for the normal spore colour of *Aspergillus niger* (Javillier, 1939; Mulder, 1948a) and *Trichoderma viride* (Brian & Hemming, 1950), and the effect of Fe concentration on pigmentation of *Torulopsis pulcherrima* (Roberts, 1946; Kluyver *et al.*, 1953), on the production of catalase (Yoshimura, 1939-'40) and vitamins—riboflavin—by moulds, yeast and bacteria (see Perlman, 1949; Lily & Barnett, 1951; Cochrane, 1958), have all been reported. The pectic enzyme activity of *Fusarium vasinfectum*, the cotton wilt pathogen, has been observed to be influenced by trace element concentrations. Increasing concentrations of Fe, Zn, Cu and Mn were inhibitory to enzyme activity, whereas growth of the fungus was not much affected (Subramanian, 1956), simulating the effect of Zn on toxin production and growth (Kalyanasundaram & Saraswathi-Devi, 1955). Some elements, apparently not required for overall growth, may still control some of the metabolic side reactions; as for instance, the essentiality of Co for production of vitamin $B_{12}$ by *Aspergillus niger* (Nicholas, 1950).

Zinc has long been considered to specifically influence oxidation of carbohydrates, suboptimal amounts resulting in an accumulation of intermediate acids (see Foster, 1949). It has later been shown, however, that suboptimal quantities of any of the other metallic elements also bring about similar incomplete oxidation (Chesters & Rolinson, 1951).

Trace element content of the medium is also known to affect the accumulation of organic and inorganic matter in fungal mycelium. Yogeswari (1948) reported B to increase dry weight (organic matter) and Zn to increase ash weight (inorganic matter) of *Fusarium vasinfectum*. The Zn amended cultures were found to have significantly higher percentages of K and Mg in their mycelium. Similar results were obtained for *Aspergillus flavus* (McHargue & Calfee, 1931).

The fact that very minute quantities of these elements suffice to meet the nutritional requirements of organisms had early evoked the assumption that they function as catalysts. Thatcher (1934),

considered Fe and Mn on the one hand and Zn and Cu on the other as 'pairs of mutually coordinating catalysts for oxidation-reduction reactions.' Modern biochemical, especially enzymological, developments have clearly shown that the trace elements function as catalysts for a number of vital enzymes. Although the latter actually perform the functions, their action is highly controlled by the presence or absence of these 'activators'. A number of enzymes isolated and characterized during recent years have been shown to need certain trace elements as vital components though not all of these enzymes have been found to occur in fungi (see Starkey, 1955). As many as twenty enzymes have been shown to be activated by Mn, which is particularly considered to be the physiologically predominant metal in the citric acid cycle (McElroy & Nason, 1954). The role of Fe and Cu in metabolism, especially in the important electron transport system of cytochromes, is well known. It would appear that Zn is necessary for the synthesis of cytochrome, at least by *Ustilago sphaerogena* (Grimm & Allen, 1954). Zinc is known to be a constituent of carbonic anhydrase, and recently two more enzymes—alcohol dehydrogenase and glutamic dehydrogenase—have also been shown to contain this element (Vallee & Hoch, 1955; Vallee *et al.*, 1955) Steinberg's (1937) assumption of the chief role of Mo in nitrate reduction, and its similarity in microorganisms and higher plants (Mulder, 1948b), have been cleary proved to be true by the later identificaton of the metal component of the enzyme nitrate reductase from *Neurospora* (Nason & Evans, 1953) and soybean leaves (Evans & Nason, 1953) as Mo (Nicholas, Nason & McElroy, 1954; Nicholas & Nason, 1955). Besides these few examples cited here, many enzyme systems are known to be activated by more than one metal (see McElroy & Nason, 1954). Discussing the general problem of multiple activation of enzymes by trace elements, Hewitt (1951) and Arnon (1952) have emphasized the difficulty of considering criteria of essentiality, when certain enzymes have been demonstrated to be activated even by non-essential elements like nickel. Nevertheless, we do know of at least certain of the vital enzyme systems specifically needing the presently recognized micronutrients (see McElroy & Nason, 1954), thus establishing their indispensability.

As with any other nutrients, trace element requirements of fungi also differ strikingly among the different organisms. Even two strains of the same species do not exhibit similar requirements for the same trace elements (Steinberg, 1919b; Nicholas, 1952; Saraswathi-Devi, 1954). Furthermore, a single organism has a

varied need for the different elements, some such as Fe and Zn being needed in larger quantities than others such as Cu, Mn or Mo. For every organism there will be certain optimal combinations of nutrient concentrations, the metabolic processes showing different trends under such combinations (see for instance, Sadasivan, 1952); that is, the requirements for each element in a medium is modified, among other environmental conditions, by the concentrations of the other elements. Indeed, these phenomena of synergism and antagonism between ions have been clearly demonstrated in some instances; for example, synergism between Mn and Ca in growth of *Marasmius epiphyllus* (Lindeberg, 1944, quoted by Lilly & Barnett, 1951), antagonism between Fe and Cu in penicillin production by *Penicillium chrysogenum* (Koffler *et al.*, 1947) and in growth of *Piricularia oryzae* (Apparao, 1959).

Before concluding, I would like to remind you all that, apart from the fundamental knowledge we gain, nutritional studies lead to an important applied aspect, *viz.*, the use of organisms for biological assays. Microorganisms in general, and fungi in particular, are well-suited as biological test organisms, because of the ease of handling, their sensitivity and specificity. Species of *Neurospora* and their biochemical mutants are widely used in vitamin assays, whereas different strains of *Aspergillus niger* are used in assay of metals (see Saraswathi-Devi, 1958b).

I have endeavoured to bring together the major trends in our knowledge regarding the two groups of micronutrients, emphasizing their physiological role. Although during recent years we have made amazing progress in understanding the mechanism of action of these substances, we have yet to go a long way before we can hope to explain, if ever, all the many enzyme activities, which these micronutrients so patently catalyze, and which constitute the mystery of life.

I have great pleasure in thanking some of my colleagues, particularly Drs. S. Suryanarayanan, D. Subramanian and M. Lakshmanan for the readiness with which they discussed various points which certainly helped a great deal in arranging my thoughts for these lectures.

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# Fractional Separation of Hydroxy and Non-hydroxy Methyl Esters of Castor Oil by Furfural and Heptane

BY

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ABSTRACT

The methyl esters of castor oil obtained by methylation of the oil have been fractionated by means of liquid-liquid extraction technique using the solvents furfural and heptane. In the present paper equilibrium solubility and phase distribution data are presented for the system castor oil methyl esters-furfural-heptane at 30°C.

## Introduction

Castor oil occupies a unique position amongst natural fats and oils in as much as unlike other fats and oils the acid components of the glycerides comprising this oil are primarily hydroxy acids. The major hydroxy acid component is ricinoleic acid which is present up to the extent of 80-90% of the total fatty acid content. The valuable medicinal and industrial uses of castor oil are largely due to this unsaturated hydroxy acid. Various methods have been adopted to fractionate the hydroxy acids of castor oil from the non-hydroxy acids either in the form of acids

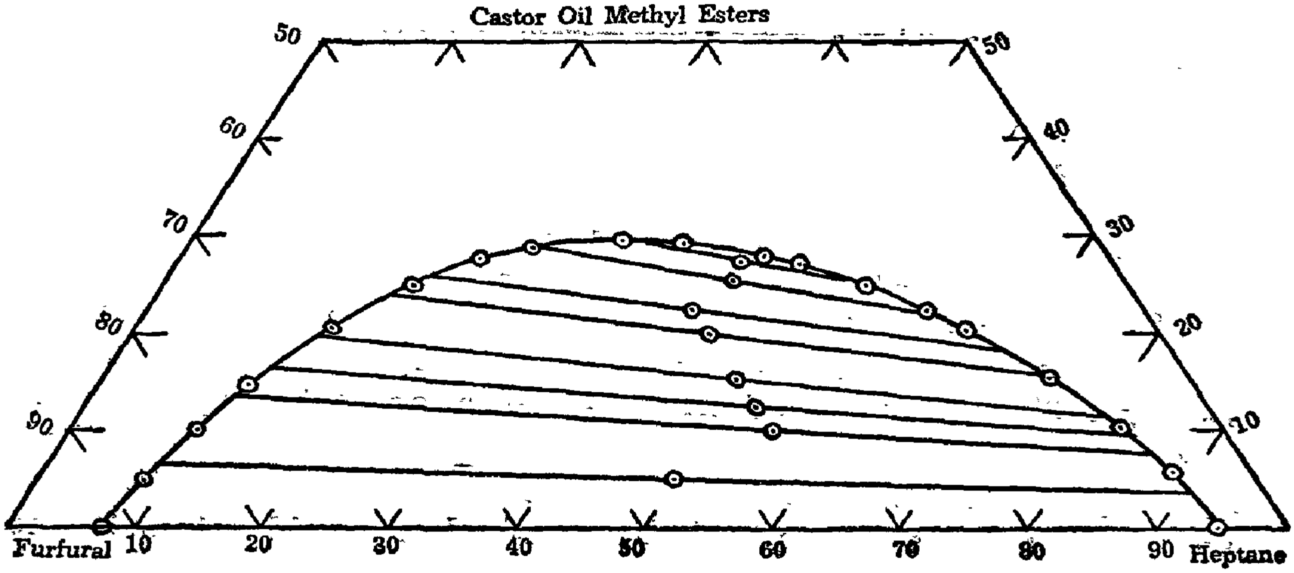


Fig. 1. Triangular Diagram
Furfural—Castor Oil Methyl Esters—Heptane System at 30°C.

or in the form of their esters. The methods such as distillation, fractional crystallisation etc. were found to be tedious and time consuming. Recently liquid-liquid extraction technique has been applied for this purpose. The application of liquid-liquid extraction technique as a means of separation of components from their solutions requires a knowledge of phase-equilibrium relationships involving mutual solubility and distribution characteristics of the constituents.

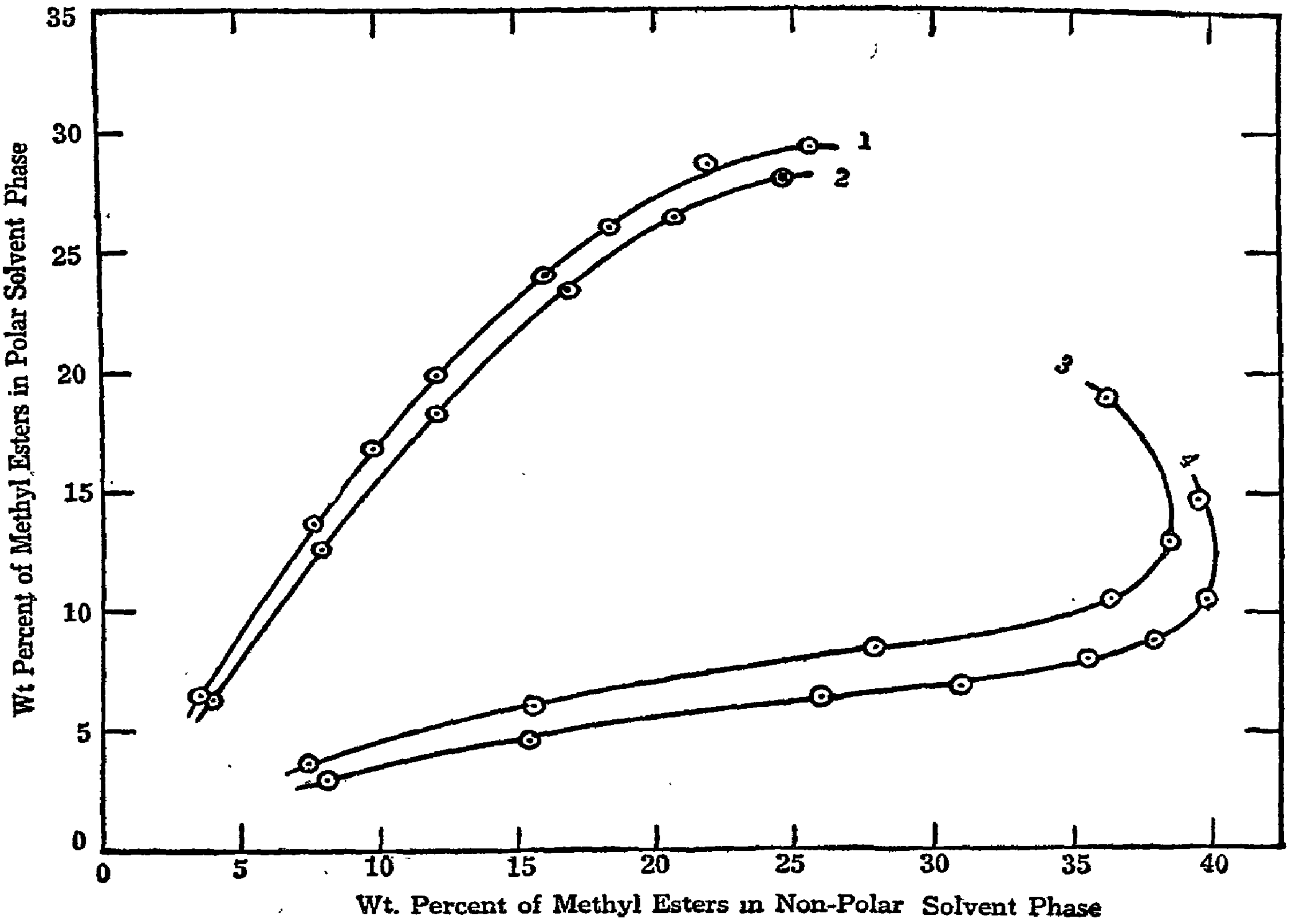


FIG. 2. Equilibrium Distribution Diagram

1. Furfural—Castor Oil Methyl Esters—Heptane System.
2. Furfural—Castor Oil Methyl Esters—Hexane System.
3. Nitromethane—Castor Oil Methyl Esters—Hexane System.
4. Nitromethane—Castor Oil Methyl Esters—Cyclohexane System.

The possibility of fractionating castor oil methyl esters by liquid-liquid extraction was first suggested by McCormack and Bolley (1954). It is based on the observation that compounds are most soluble in solvents which they resemble structurally. Thus hydroxy acid esters of castor oil could preferentially be distributed in the polar solvents and the non-hydroxy acid esters in the non-polar solvents.

In order to facilitate the extraction the solvents should be immiscible or partially miscible with each other and the esters must be completely miscible in each of the solvents. Hence it is desirable that the critical solution temperature of the solvent pairs

TABLE — I

Critical Solution Temperatures of Solvents

| Pair of Solvents | | | Critical solution |
|---|---|---|---|
| Polar | Non-polar | | Temperature °C |
| Furfural | n-Heptane | .. | 95 |
| Furfural | n-Hexane | .. | 92 |
| Furfural | Cyclohexane | .. | 69 |
| Nitromethane | n-Hexane | .. | — |
| Nitromethane | Cyclohexane | .. | 79 |
| Methanol | n-Heptane | .. | 51 |

should be high, since higher critical solution temperature signifies that solvents are only partially miscible at lower temperatures. The two solvents should have appreciable difference in boiling points and densities for easy separation. A priliminary study was made of the various solvents miscible with castor oil methyl esters but partially miscible with each other at 30°C. The pairs of such solvents are presented in Table I along with their critical solution temperatures.

The dielectric constants of these solvents presented in Table II, show that furfural, nitromethane and methanol are highly polar

TABLE — II

Dielectric Constants of Solvents

| Solvent | | Dielectric Constant | Temperature °C |
|---|---|---|---|
| Furfural | .. | 41·90 | 20 |
| Nitromethane | .. | 35·87 | 30 |
| Methanol | .. | 32·63 | 25 |
| n-Hexane | .. | 1·89 | 20 |
| n-Heptane | .. | 1·92 | 20 |
| Cyclohexane | .. | 2·02 | 20 |

compared to the solvents hexane, heptane and cyclohexane which have very low dielectric constants.

The extraction of methyl esters of castor oil by the pair of solvents methanol and heptane was studied by McCormack and Bolley (1954). As the pair has low critical solution temperature the maximum concentration of the mixed esters in the system is only 8·5% by weight even at 24°C. Kasturirangan and Laddha (1955) studied the system furfural-castor oil methyl esters-cyclohexane at 30°C. Later fractionation of methyl esters of castor oil using solvent pairs nitromethane and cyclohexane (1959), nitromethane-hexane and furfural-hexane (1960) at 30°C. was studied by the present authors. This paper presents the equilibrium distribution data for the system castor oil methyl esters-furfural-heptane.

## Experimental

*Materials used :*

1. Castor oil methyl esters: These were prepared by methylation of castor oil as suggested by Brown and Green (1940). The mixed esters used had the following properties: Density $d^{30}_4$ 0·9250: Refractive index at 30°C 1·4602: Specific rotation $\alpha^{30}_d$+5·4: Hydroxyl value 165·75.

2. Furfural: Furfural supplied by General Chemical Division, Allied Chemical and Dye Corporation, N.Y., was purified by vacuum distillation each time just before use. The properties were: Density $d^{30}_4$ 1·124 and refractive index at 30°C. 1·5184.

3. Heptane: n-Heptane supplied by B.D.H. Ltd., was fractionated over a rectification column and the fraction between 97·5 and 99·5°C was collected. The physical constants were: Density $d^{30}_4$ 0·7345 and refractive index at 30°C 1·3930.

Experimental Procedure :

The limiting solubility and tie-line data for the ternary system furfural-Castor oil methyl ester-heptane at 30°C were determined by the method described by Othmer et al (1941). The procedure has been discussed in detail by the authors previously (1959). The extent of fractionation of hydroxy and non-hydroxy esters of castor oil distributed in the polar and non-polar solvent phases was determined by estimating the hydroxyl values of the extracted

esters from the polar and non-polar solvent phases by the pyridine-acetic anhydride method described by Burton and Robertshaw (1939).

## Results

The equilibrium solubility and phase distribution data along with the hydroxyl value of methyl esters distribution in the furfural and heptane phases are given in Tables III and IV respectively. The data are represented in Fig. 1 by plotting the ternary

TABLE — III

Equilibrium solubility Data at 30°C for the System Castor Oil Methyl Esters-Furfural-Heptane

| Weight Percent Composition of Components | | |
|---|---|---|
| Furfural | Methyl Esters | Heptane |
| 92·50 | 0·0 | 7·5 |
| 86·65 | 4·88 | 8·4 |
| 80·07 | 9·93 | 10·00 |
| 73·58 | 14·96 | 11·46 |
| 64·20 | 20·21 | 15·59 |
| 55·35 | 24·92 | 19·73 |
| 48·99 | 27·62 | 23·38 |
| 44·67 | 28·54 | 26·79 |
| 40·59 | 29·46 | 29·95 |
| 36·92 | 29·44 | 33·64 |
| 32·02 | 28·84 | 38·64 |
| 27·21 | 27·87 | 44·92 |
| 24·51 | 27·40 | 48·09 |
| 20·11 | 24·89 | 54·99 |
| 16·77 | 22·36 | 60·87 |
| 14·58 | 20·33 | 65·08 |
| 10·57 | 15·65 | 73·78 |
| 7·69 | 10·21 | 82·10 |
| 5·55 | 5·69 | 88·76 |
| 4·98 | 0·0 | 95·02 |

phase distribution data on the conventional triangular chart, considering castor oil methyl esters as a single component in the ternary system. The hydroxyl values of the esters in the furfural phase at lower concentrations of methyl esters in the original mixture, are comparable with the hydroxyl value of pure methyl ricinoleate, which is 179·5, as in the case of other systems. The distribution curves for the esters using the different solvent pairs are presented in Fig. 2. The plot indicates that the weight distribution of the esters favours furfural phase in the case of furfural-hydro-

TABLE — IV

Equilibrium Tie Line Data at 30°C for the System Castor Oil Methyl Esters — Furfural — Heptane

| Composition of mixture taken | | | Weight percent of esters in | | Hydroxyl values of esters | |
|---|---|---|---|---|---|---|
| Esters | Furfural | Heptane | Furfural phase | Heptane phase | Furfural phase | Heptane phase |
| 5·0 | 45·05 | 49·95 | 6·55 | 3·51 | 186·5 | 128·6 |
| 10·05 | 35·00 | 54·95 | 13·65 | 7·65 | 185·3 | 142·5 |
| 12·50 | 35·00 | 52·50 | 16·80 | 9·56 | 185·3 | 141·3 |
| 15·35 | 35·00 | 49·65 | 19·83 | 11·84 | 182·0 | 146·2 |
| 19·95 | 34·95 | 46·11 | 24·09 | 16·06 | 179·0 | 148·4 |
| 22·38 | 35·04 | 42·58 | 26·11 | 18·31 | 176·3 | 152·1 |
| 25·55 | 30·46 | 44·09 | 28·87 | 21·97 | 168·1 | 160·7 |
| 27·65 | 28·27 | 44·02 | 29·42 | 25·69 | 167·2 | 164·4 |

carbon systems and the hydrocarbon phase in the case of nitromethane-hydrocarbon systems. However, the mixed esters get fractionated and the hydroxy esters are prominently present in the polar solvent phase in both the cases, furfural and nitromethane being the polar solvents, as evidenced by the determination of the hydroxyl values of esters obtained from the polar and non-polar solvent phases. The study also indicates that when the polar solvent concentration is kept constant and the concentration of the esters is increased in the mixture taken for phase separation, the

hydroxyl value of the sample obtained from polar solvent layer is decreased. The plait point compositions, as shown in Table V,

TABLE — V

Plait Point Composition

| | Plait Point Compositions | | |
|---|---|---|---|
| Systems | %A | %B | %C |
| Furfural (A)-Hexane (B)-Esters(C) .. | 29·4 | 43·4 | 27·2 |
| Furfural (A)-Heptane (B)-Esters (C) .. | 28·4 | 43·0 | 28·6 |
| Nitromethane (A)-Hexane (B)-Esters (C) .. | 64·9 | 10·9 | 24·2 |
| Nitromethane (A)-Cyclohexane (B)-Esters (C) .. | 64·8 | 10·2 | 25·0 |

indicate that the percentage of esters present at plait point condition is the greatest in the case of furfural-methyl ester-heptane system as compared to other systems sudied earlier. This is expected since furfural-heptane pair has the highest critical solution temperature as compared to other solvent pairs.

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# *Didymozoon Tetragynae:* A Digenetic Trematode of the Family Didymozoidae

BY

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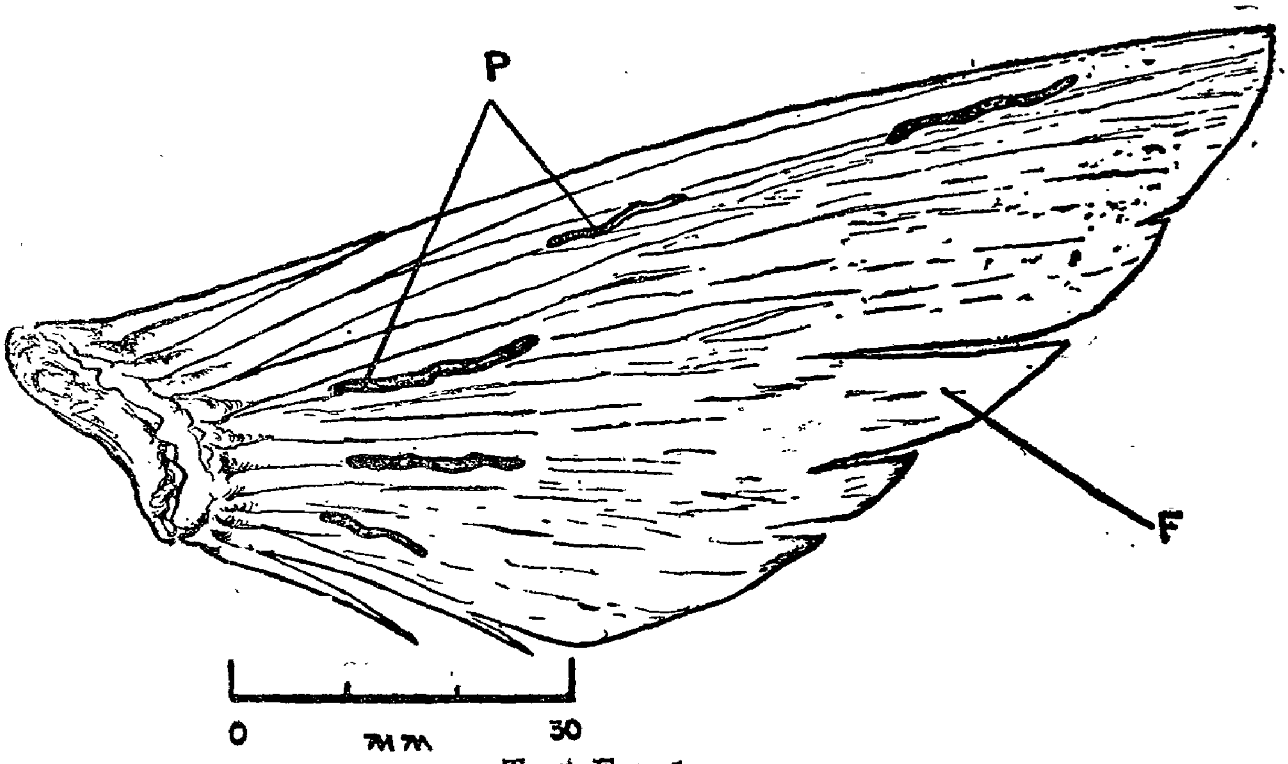
## INTRODUCTION

Several parasites of the family *Didymozoidae* parasitizing three species of fish of the genus *Sphyraena* (Barracuda) were obtained from the sea off Rameshwaram island, Tuticorin, Porto-novo, Madras and Andaman islands. This record thus extends the distribution of the Parasites to the Indian region from the Mediterranean on the one hand and the Japanese (Pacific) region on the other. Two new species *Didymozoon apharyngi* (Job, 1961a) and *Platocystis polyastra* (Job, 1961b) have been described from this




TEXT-FIG. 1.

collection covering two distinct genera *Didymozoon* and *Platocystis* respectively. The present form undoubtedly belongs to the

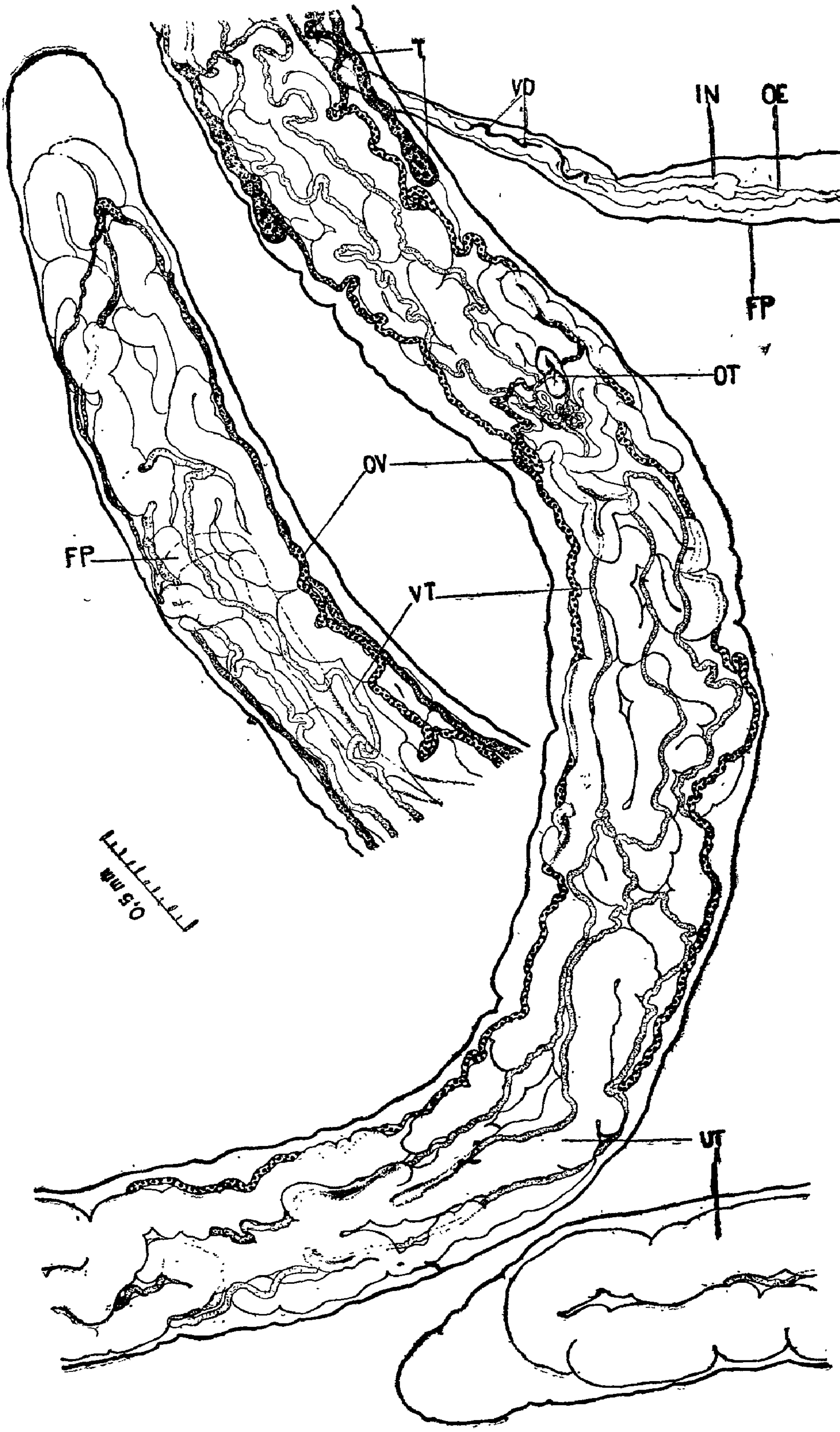
former genus and is very closely allied to the former species as well; but differs in some important details. The body proportions are generally greater, the fore body being exceptionally long and club tipped and the ovary branched into four arms forming the pattern of the letter *H*. Hence the following detailed description of the form is presented with reasons for assigning it to the status of a new species.

### Genus **Didymozoon** TASCHENBERG, 1878

The following description is summarised from Yamaguti 1957, in order to bring out the salient features shared by the present form. Parasites on fish encysted in pairs; completely hermaphrodite; body divided into a slender forepart (fore body) and a thicker hind body, straight and cylindrical; fore body attached to hind body sub-terminally oral sucker Present; no acetabulum; testis paired, elongate, at anterior part of hind body; vas deferens not forming definite seminal vesicle; no pars prostatica; genital pore beside oral sucker; vitellarium branched narrow and winding more extensively; uterus occupying almost entire available space of hind body; ovary occasionally branched.

### **Didymozoon tetragynae** n.sp.

As illustrated in Figure 1, these were found encysted mostly on the paired fins of *Sphyraena picuda* and *S. jello*. The cysts are long, straight and cylindrical measuring between 12 and 20 mm. This really refers to the length of the hind body. It is *ca* 0·68 mm. thick and tapers into a blunt point at both ends. The fore part is very slender except near the free end where it becomes club shaped. At its thinnest part it is 1/6th and at its thickest 1/3rd the thickness of the hind body. The fore part is exceptionally long and measures nearly half the length of the hind body. It stems from the hind body subterminally at a point 1/10th of the total length from the anterior end. The oral sucker is roundish measuring *ca* 0·18 mm. along the shorter antero-posterior axis and 0·20 mm. along the lateral axis. The only feature it shares with *D. apharyngi* is the absence of a pharynx. The intestinal caeca commence a little behind the oral sucker at a point nearly 1/6th of the total length of the fore body. Testis paired, nearly 4/5th the length of the fore body and is also club shaped. The ovary is very long with four branches extending anteriorly and posteriorly from the ootype in the form of the letter '*H*', the length of the two




TEXT-FIG. 2.

anterior or the two posterior arms being alternately long and short. The ovary and vitellaria enter freely into the testicular zone. The ootype is located almost in the middle of the hind body, closer to the base of the fore body and is eliptical in shape measuring *ca* 25μ × 8μ. The eggs are half moon shaped more angular and measuring about 1·5μ × 1·0μ.

The present form is similar to *D. apharyngi* in the absence of the pharynx and the smaller of the parasites could easily be mistaken for that species in general appearance. But in the pattern of branching of the ovary primarily and in the extremely long and filamentous fore body in the rounded oral sucker and in most of the body proportions mentioned above the present form is distinctly different, and offers sufficient ground for naming the parasite (on the basis of the first mentioned character) *Didymozoon tetraqynae* and assign it tentatively to the status of a new species. The holotype will be deposited in the collection of the Madras University Zoology Research Laboratory.

ACKNOWLEDGEMENTS

The author is indebted to his former colleague Mr. G. Luther for his help in the collection and identification of the host species and to his colleague Dr. K. Ramalingam for valuable suggestions.

EXPLANATION OF ABBREVIATIONS USED IN TEXT-FIGS:

F.—Fin; GA.—Genital aperture; IN.—Intestine; M.—Mouth; OE.—Oesophagus; OS.—Oral sucker; OT.—Ootype; OV.—Ovary...P. Parasites; T.—Testis; UT.—Uterus; VD.—Vas deferens; VT.—Vitellarium.

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# Cell Divisions in the Shoot Apices of *Sarcandra irvingbaileyi*

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SUMMARY

Divisions in the cells restricted to the vacuolated zone of the vegetative apices of *Sarcandra irvingbaileyi* have been presented to show that growth is perceptible in this zone. Thus, the inferences obtained through this study does not lend support to the inactive meristem ('méristème d'attente') concept of the french school of workers.

The inter-relationship of the various zones of the shoot apex of *Sarcandra* has already been described in an earlier paper (Ramji, 1961). To summarise briefly: the shoot apex at the maximal phase of plastochron (Plate I, Fig. 1) consists of a tunica of two layers with the corpus below, which is divisible into a central mother cell zone with the radiating zone on either side (as viewed in median longisections) and the rib meristem subjacently. A group of cells restricted to the summit of the apex and lying in the tunica and corpus tends to stain differentially with cytoplasmic and nuclear stains and appears vacuolated when compared to the neighbouring cells (Plate I, Fig. 1). During recent years this vacuolated cell group has been reported in a larger number of dicotyledonous and gymnospermous shoot apices (for citations refer Gilfford, 1954). Recently however, a few French workers (Buvat, 1951a, 1952 and 1955; Lance, 1953) have advanced an interesting viewpoint by attributing a total inactivity to this zone of vacuolated cells during the vegetative cycle of an apex. The opinions of this french school of workers have been tested with reference to the present material, the results of which analysis are presented here.

## MATERIALS AND METHODS

The sources for materials and the methods followed were the same as detailed in an earlier publication (Ramji, 1961).

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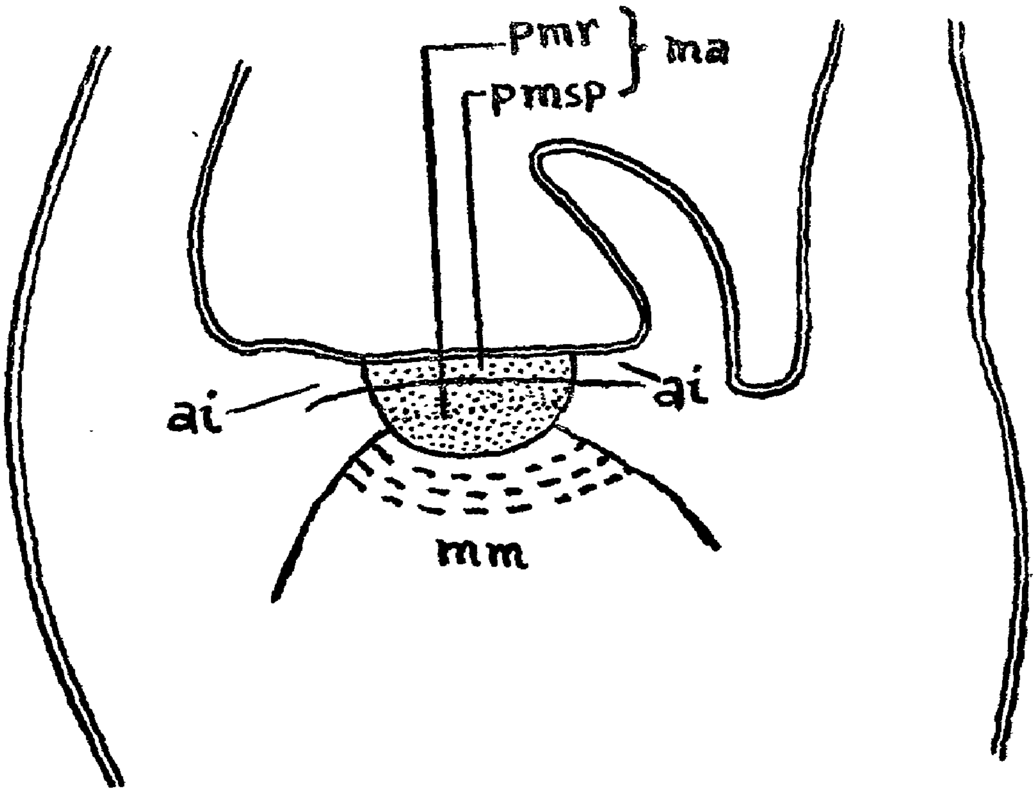
## Observations

At the maximal phase of plastochron, the vacuolated cell group is restricted to a few cell layers at the extreme summit of the vegetative apex (Plate I, Fig. 1). This cup-shaped zone will corres-




Text-Fig. 1: The shoot apex of *Myosurus minimus* (reproduced after Buvat, 1951 a)

*pmsp*— 'proméristème sporogène' } —*ma*—'méristème d'attente'
*pmr*— 'proméristème recéptaculaire' }
*mm*— 'méristème medullaire'
*ai*— 'anneau initial'

pond to the 'méristème d'attente' described by Buvat (1951a, 1952). It is in this group that Buvat (1952) opined that mitoses are rare and this group functions only during the reproductive cycle of the plant (Buvat, 1951b). However, during the present investigation, cell divisions are observed to be persistent in this vacuolated cell group as in the surrounding region during the vegetative cycle of the apex.

Plate I, Figure 1 represents the maximal phase of plastochron of the apex, while in Plate I, Figure 2, the arrow indicates an oblique division (which may eventually become anticlinal) in a cell at the extreme summit of the apex. The position of this cell is in the region of vacuolation.

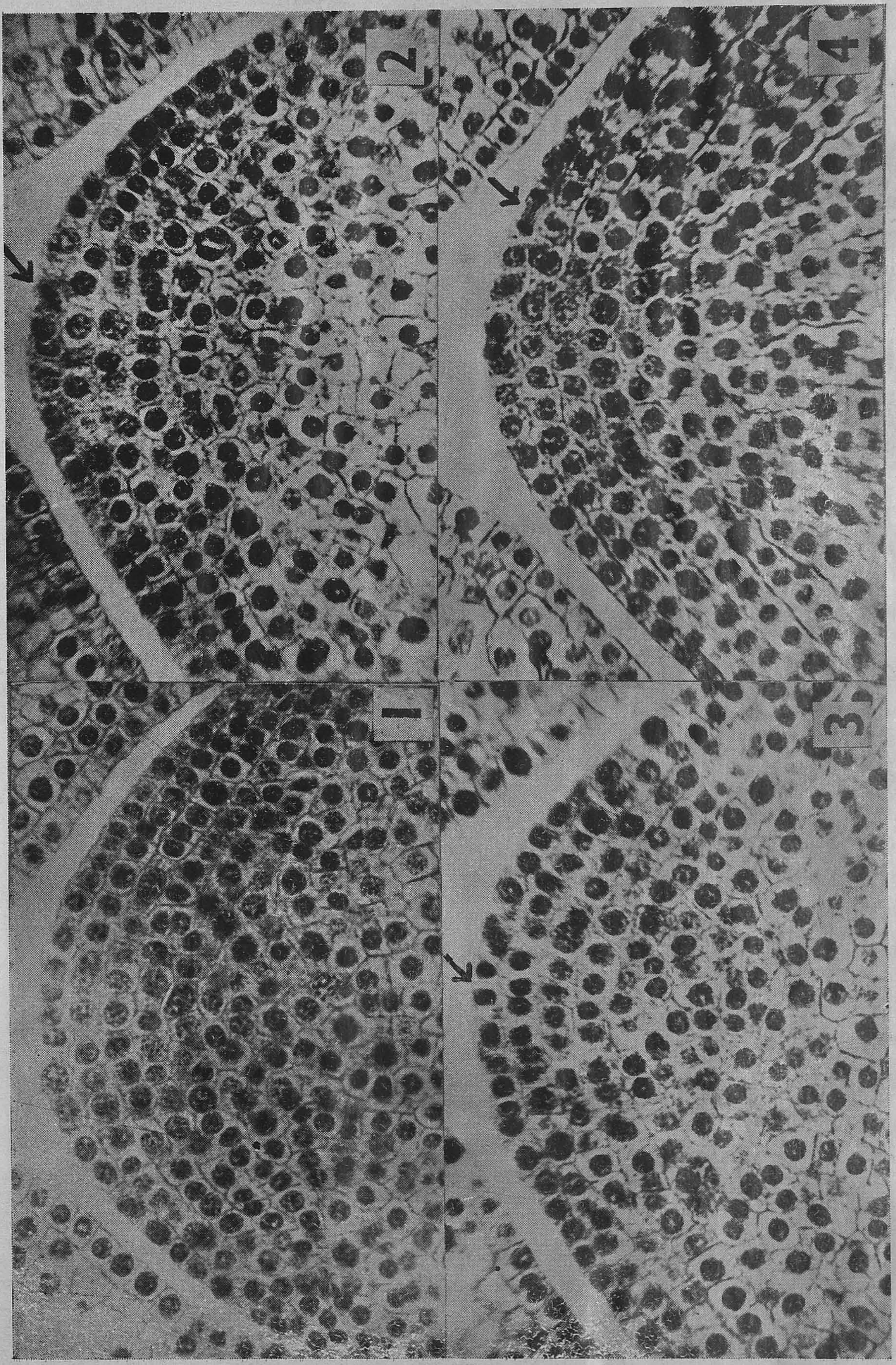



FIG. 1. Median longitudinal section of a vegetative apex of *Sarcandra* during maximal phase of plastochron showing the tunica-corpus alignment and the presence of the cup-shaped vacuolated zone at the summit of the apex. × 490 FIGS. 2, 3 & 4. Median longisection of the shoot apex towards maximal phase of plastochron. Arrow indicates the dividing cell. FIG. 2. × 490; FIGS. 3, 4 — × 450.

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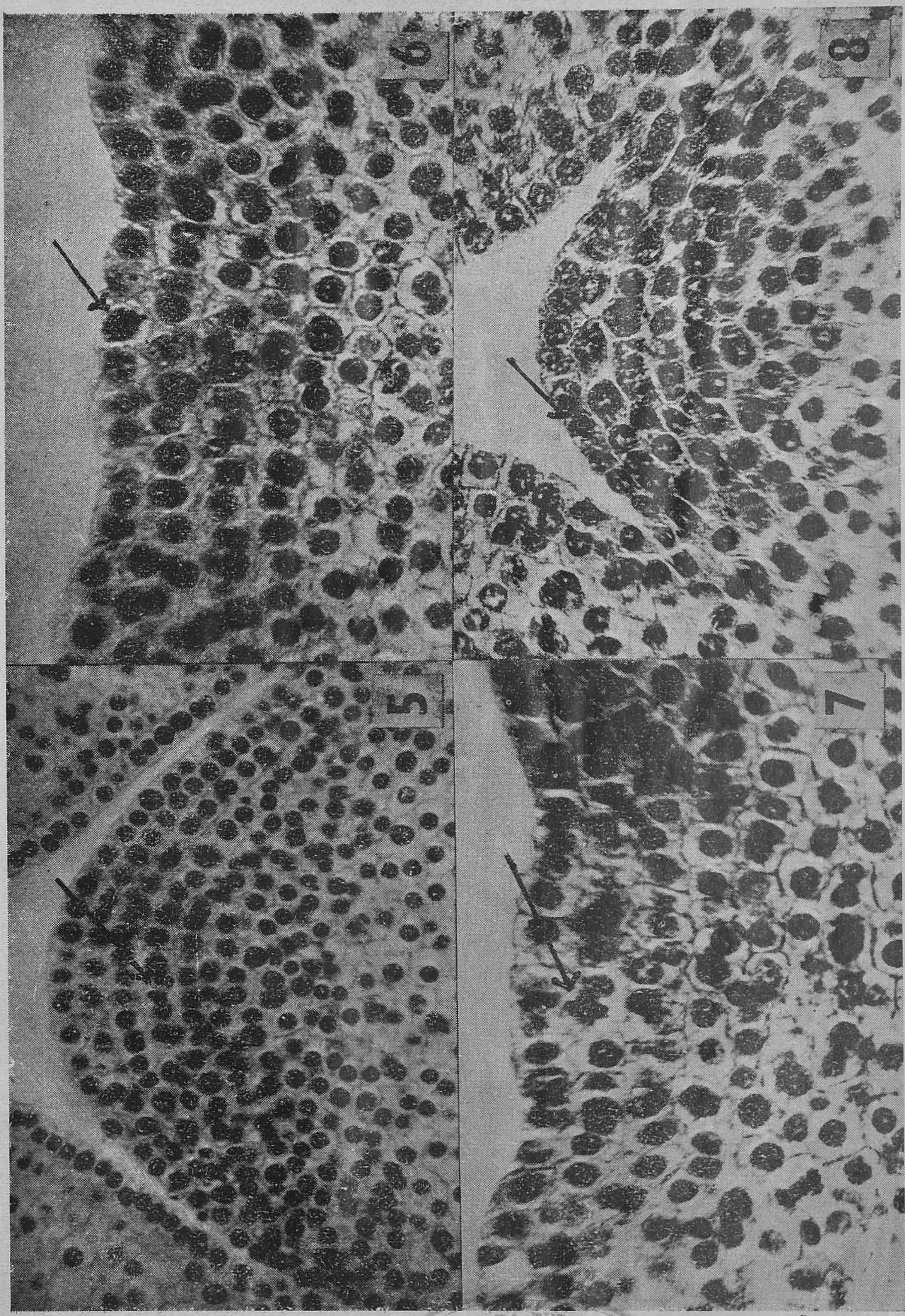


Fig. 5. L.S. of the shoot apex towards maximal phase. Arrow indicates a cell of the corpus lying in the region of vacuolation in division. × 390. Figs. 6 & 7. L.S. of the apex during post-leaf initiation phase. Arrow indicates the dividing cell. × 510. Fig. 8. L.S. of a shoot apex that is resolving from the minimal phase to the maximal. Arrow indicates the site of division. × 510.

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In Plate I, Figure 3, arrow indicates the two daughter cells after a recent division in a cell lying in the vacuolated region of the apex. Similarly, a cell on the flanks of the outer tunica layer and lying in the region of vacuolation is in division in an anticlinal plane (Plate I, Fig. 4). Plate II, Figure 5 is yet another stage indicative of nuclear division in a cell of the corpus which in turn is in the vacuolated region of the apex.

All the above figures represent stages obtained more or less during the maximal phase of the plastochron. While this is so, during minimal phase, it was very common to notice numerous division figures in the cells of the apex. Plate II, Figures 6 and 7 are representatives of such a phase when nuclear divisions are noticed in the cells at the summit of the apex; in the former figure, a cell in the outer tunica layer is in division while in the latter, a cell in the inner layer is dividing anticlinally. Plate II, Figure 8 is of an apex during the resolution of the same from the minimal phase to the maximal phase of plastochron. Arrow indicates the site of nuclear division in the cell situated toward the summit of the apex. Also, the phenomenon of vacuolation is lacking at this phase of development of the shoot apex, thus pointing out that this feature appears short-lived and is clearly restricted to the maximal phase of the shoot apex (compare Plate I, Fig. 1 and Plate II, Fig. 8).

All the above figures show that cell divisions occur in the summit of the apex and bring about growth of the vegetative apex as a whole.

## Discussion

The presence of a vacuolated zone restricted to the summit of the shoot apex has been reported for many angiosperms (for citations refer Gifford, 1954, p. 493). The phenomenon of vacuolation in the axial apical region of the shoot apex seems variable during different stages of growth of an apex (Ramji, 1961 and the present study) or may be totally lacking in an apex (Tepfer, 1960). Hence this character seems to be a (transitory) feature, probably well expressed during the maximal phase of a plastochron. Based on this phenomenon, recently the french school of workers (especially Buvat, 1951a, 1952) opines that the apical axial region of the vegetative apex is inactive (the 'méristème d'attente' of Buvat; also see Gifford, 1954, pp. 486-491 for fuller details regarding the above concept).

Reproduced in Text Figure 1 is the schematic representation of the vegetative apex after Buvat (1951a). According to him (1951a) the apical axial region of the vegetative apex of *Myosurus minimus* is inactive during the vegetative cycle. This zone is said to become active during the reproductive phase i.e. during the time of flowering. In another paper (Buvat, 1951b), it has been stated that the apical axial region of the apex (the 'méristème d'attente') transforms and builds up the floral receptacle while the 'anneau initial' ceases to produce the leaf and disappears. His views are based on the plotting of division figures from serial longi- and transections of the vegetative apex. This 'méristéme d'attente' concept of Buvat, as has been pointed out by Gifford (1954), seems to be an extension of the 'anneau initial' (or the initial ring) concept of Plantefol (1947) and is supported by the works of Lance (1953, 1957). In *Vicia faba,* Lance (1953) says that "the statistical distribution of mitoses, realised by means of superimposition of the transversely and longitudinally cut sections, shows that the apical axial region is entirely deprived of division and this is in contrast to the interior of the meristematic mantle, corresponding in its upper part of the 'anneau initial' described by Plantefol."

Gifford (1954) has already cited a few objections to the total acceptance of the theories of Plantefol, Buvat and their colleagues. Some further points of criticisms have also been put forth and the subject has received a careful review recently (Wardlaw, 1957; Cutter, 1959). Recently, Paolillo and Gifford (1961) have produced further evidences that in the shoot apices of *Ephedra,* the sub-apical initiation zone is active and yields derivatives to the subjacent tissues of the apex. This is in contrast to the views of Buvat who includes the gymnospermous shoot apices also under his concept. Also, the series of publications of Wardlaw (1956), Wardlaw and Cutter (1955, 1956) have shown that in *Dryopteris aristata,* the growth occurs in the distal region of the apical meristem, rather slowly, as compared with the other regions of the apex. Even though Buvat has not included the apices of ferns under his concept, these studies show that the inactive meristem concept of Buvat does not seem to hold true for gymnosperms as well as ferns.

In the present investigation, the series of photographs have been so chosen to show mitotic activity obtained more or less in the vacuolated region (the 'méristème d'attente') of the apex during different plastochronic stages. Considering Buvat's concept again, the vacuolated axial region of the shoot apex does not

undergo any growth during the vegetative phase and growth in the apex is centred largely in the peripheral regions i.e. the 'anneau initial'. A comparison of Plate I, Figure 1 and Plate II, Figure 8 of the present paper shows that this region of vacuolation so well expressed at the maximal phase (Plate I, Fig. 1) is totally lacking in Plate II, Figure 8. Then the question naturally arises: what has happened to this vacuolated zone? Since it is not expressed during the post-minimal phases of plastochron, the region of vacuolation represents only a stage in the ontogeny of the shoot apex of *Sarcandra,* and to attribute any kind of total inactivity to this region seems highly incongruous to the present writer. Further, the present investigation reveals that due to divisions in the cells of the vacuolated zone, an increase in the volume of the shoot apex is caused. It seems pertinent to point out the present writer's observations on the seedling and adult apices of *Calophyllum inophyllum.* In this plant the region of vacuolation, so characteristic of the adult apices, is totally lacking in the early stages of growth of the seedling. When this is so, how is it possible to reconcile with a differential growth pattern in the vegetative apex on the basis of a transitory phenomenon? Recently, Clowes (1959) has shown that the vacuolated zone of cells in the vegetative apices of *Coleus* cannot be considered as inactive as in the root apices. So also Ball's (1960) observations on the living shoot apices of *Asparagus* and *Vicia* have proved that cell divisions occur in the axial part of the apex as in the peripheral regions, negating the possibility of any inactive meristem restricted to the tip of the apex. However, it should be pointed out that the time involved to complete a nuclear division varies between the apices of the above two plants (Ball, 1960). It is also desirable to point out here that the opinions of Ball (1960), who disapproves the data collected from studies on cell division in the shoot apices of killed, fixed and processed material, are incompatible with the views of the present writer. It is true that the various fixatives used and the process of microtomy may induce patterns in cell walls not originally laid down. But how far the fixatives used (usually Craf III and FAA) obscure the presence of cell divisions in the vegetative apex is still not clear. Also it should be remembered that any culture media for the growth of the materials concerned vary and contain all the essential growth promoters and regulators that influence growth. But do such results obtained through the artificial growth medium of culture solutions or alike reflect *truly* the changes that occur under natural conditions (which is varaible in it-

self)? This should be determined before one proceeds to place absolute emphasis on these results. Until then data collected from fixed and processed material will serve a useful purpose to understand the phenomenon of growth in the apex. One conclusion that seems highly possible from such studies of Buvat and his colleagues is that the region of very pronounced activity is on the lateral sides of the vegetative apex as has been reported by other workers (*cf.* Wardlaw, 1957, p. 227 for complete citations).

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# On the Experimental Determination of the Activation Energy of a Chemical Reaction

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ABSTRACT

It is shown that the activation energy of a complex chemical reaction could be determined by plotting log $(-\triangle c/\triangle t)$ against 1/T. where, for a given initial concentration, $-\triangle c$ denotes a small and constant fraction of the reaction at the various temperatures. The advantages of this method are discussed.

The activation energy ($E$) of a chemical reaction is generally determined by plotting log $k$ against $1/T$, where $k$ is the specific rate constant and $T$ is the absolute temperature. The values of $k$ needed for the plot are usually obtained from one of the following well known equations.

$$k_1 = \frac{1}{t} \ln \frac{a}{a-x} \qquad .. \quad (1)$$

$$k_2 = \frac{1}{t} \cdot \frac{x}{a(a-x)} \qquad .. \quad (2)$$

$$k_3 = \frac{1}{2t} \left\{ \frac{1}{(a-x)^2} - \frac{1}{a^2} \right\} \qquad .. \quad (3)$$

where $k_1$, $k_2$, and $k_3$ are the first, second and third order rate constants respectively, $a$ is the initial concentration and $x$ is the amount reacted in time $t$. For simplicity, only cases where the order of the reaction ($n$) is a small whole number are considered

here. The equations given above are also in their simplest forms. In this paper it will be shown that a simpler procedure is possible to evaluate $E$. The method suggested is applicable, especially, to reactions for which the experimental values of $n$ are fractional and somewhat uncertain.

When experimental data corresponding to a single initial concentration are available the order of the reaction is determined by finding out the equation into which the data fit in best. If this procedure is adopted at different temperatures also, reliable values of $k$ can be obtained for an Arrhenius plot. However, this method fails to reveal the value of $n$ in the case of complex reactions such as, for example, the halogenation of aromatic compounds in solution. (Robertson, De La Mare and Johnston, 1943; Lambourne and Robertson, 1947; Ganesan, 1953; Yeddanapalli and Gnanapragasam, 1956). Kinetic studies of these reactions are complicated by the occurrence of the process

$$X_2 + HX \rightleftharpoons HX_3$$

where X denotes a halogen atom. Due to this side reaction, rate constants calculated by utilising the above equations fall off rapidly during the course of the halogenation reaction. Therefore the orders of such reactions have been determined by measuring the rates in the early stages of the reaction. The value of $n$ is evaluated from the equation

$$\frac{t_1}{t_2} = (a_2/a_1)^{n-1} \qquad \text{.. (4)}$$

where $t_1$ and $t_2$ are the times taken for the completion of a small fraction (say, 10%) of the reaction at two different initial concentrations $a_1$ and $a_2$, respectively. The values of $t_1$ and $t_2$ are obtained from plots of percentage decomposition ($100x/a$) against time ($t$).

After determining the value of $n$, the activation energy has been evaluated as follows (Robertson *et al.*, 1943; Yeddanapalli *et al.*, 1956). For a given initial concentration and at various temperatures, graphs of $100x/a$ against $t$ are drawn. Values of $t$ for the initial 10% (or any other convenient amount) transformation are read from the graphs. Then, by making use of the appropriate rate equation, the rate constants corresponding to the 10% change

are calculated for the various temperatures. Utilising these values of $k$, Arrhenius plots are drawn.

Although the activation energies obtained by this method may be quite accurate, it is to be noted that the values of $k$ have little significance by themselves. For instance, let us consider a third-order reaction for which $k_3$ has been calculated by means of equation (3). It can readily be seen that, at different temperatures,

$$k_3 \propto 1/t \quad \text{.. (5)}$$

when $a$ and $x$ are constant; that is, the values of the rate constants in the Arrhenius plot specified above satisfy equation (5). Hence *it follows that a plot of log (1/t) against 1/T is sufficient for evaluating E.* Moreover, equations (1) and (2) also reduce to (5) when $a$ and $x$ are constant. This simplification leads us to the conclusion that, for a reaction whose order has been found (by measuring the initial rates) to be 3, the Arrhenius plot specified above need not be of the form log $k_3$ versus $1/T$ and that the log of any rate constant could be plotted against $1/T$. The different graphs will all give the same value for $E$.

The question now arises whether it is necessary to determine the order of a complex reaction, however indirect or limited in scope the method employed may be, before evaluating the activation energy of the reaction. The halogenation reactions, given as examples already, have all been found to be either second or third order, most of the experimental values of $n$ being very close to 2 and 3, respectively. In some cases values such as 2·3; 2·7 and 3·4 were also obtained for $n$. Moreover, even in the case of a single reaction the order sometimes changed from 3 to 2 when the experimental conditions, such as concentration, were altered. The mechanism proposed for a gas-phase reaction is usually a summation of several elementary reaction steps and, according to Steacie (1954), too much importance should not therefore be attached to the exact value of $n$, which happens to hold over a narrow range of experimental conditions. A somewhat similar situation may exist in the case of reactions in solution also. An experimental value of 2·5 (or even 2·3, 2·7, etc.) for the order of a reaction may simply mean that the over-all reaction consists of a second-order and a third-order process. The activation energies of such complex reactions could be determined by the method outlined below.

The rate of any chemical reaction can be expressed by

$$-\frac{\Delta c}{\Delta t} = kc^n \qquad \ldots \quad (6)$$

where c is the concentration. $k$ varies with temperature according to the equation

$$k = Ae^{-E/RT}. \qquad \ldots \quad (7)$$

From equations (6) and 7 we get

$$\ln(-\Delta c/\Delta t) = \ln A - \frac{E}{RT} + n\ln c \qquad \ldots \quad (8)$$

For a given reaction, $n$ may be either integral or fractional but is a constant. If the reaction is studied at a single concentration at different temperatures, if $-\Delta c$ denotes a constant extent of the reaction and if both $-\Delta c$ and $\Delta t$ are reckoned from the start of the reaction, then equation (8) reduces to

$$\ln(1/t) = -\frac{E}{RT} + \text{constant.}$$

Hence a plot of log $(1/t)$ against $1/T$ will give $E$ (cf. equation 5).

In some kinetic studies, where the experimental techniques are somewhat tedious, it may not be possible to obtain by the usual graphical method precise values of $t$ corresponding to a constant value of $-\Delta c$. In such cases the reaction times at the various temperatures could be so adjusted as to maintain $-\Delta c$ as constant as possible (e.g., $10 \pm 1\%$). $E$ could then be evaluated by plotting log $(-\Delta c/\Delta t)$ against $1/T$ (refer equation 8).

In the above method and also in the one adopted earlier (Robertson *et al.*, 1943) $-\Delta c$ is maintained constant at different temperatures. But the actual Arrhenius plot is simpler in the present method. In the halogenation reactions the real values of $k$ are not known. At each temperature the value of $k$ falls off during the course of the reaction. In other words, the error introduced in the real value of $k$ varies with the extent of the reaction. However, when $-\Delta c$ is constant and small, the percentage error in the values of $k$ will, to a first approximation, be

constant at different temperatures. Any Arrhenius plot drawn under these conditions will therefore give the most accurate value possible for $E$.

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